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AN INVESTIGATION OF THE EFFECT OF *GAMMEXANE* IN A HALF-BATH ON HARD TICKS ON CATTLE

By

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INTRODUCTION

The urgent need for insecticides as effective as derris and pyrethrum but cheaper to produce, led to the discovery of the insecticidal properties of benzene hexachloride (B.H.C.). It was found in 1943 that the insecticidal properties were vested chiefly in the gamma isomer of the compound, which was given the name "Gammexane" (Slade, 1945). Subsequent work on this substance showed it to be effective against many species of insects, arachnids and some crustacea. Laboratory tests proved that the gamma isomer of benzene hexachloride in very low concentrations kills hard ticks and manufacturers proceeded to make up the substance into cattle dips.

In Tanganyika, the main stock routes pass through many areas in which East Coast fever is enzootic. During 1939-45 the movement of cattle along the stock routes was greatly increased and it became important that animals should be protected against vector ticks during their trek. At certain strategic points along the routes dipping tanks were erected, but because of war-time conditions and the need of economy, it was not possible to build sufficient to protect the cattle against ticks throughout the whole of their journey and hand-dressing stations were therefore interposed at certain points. At these the ears, polls, tails and perineal regions of the cattle were treated with an oily dressing, which gave a limited measure of protection.

Dr. S. G. Wilson, of the Veterinary Laboratory, Entebbe, Uganda, suggested the use of a "half-bath" containing a gammexane wash through which the animals could walk without being totally immersed.

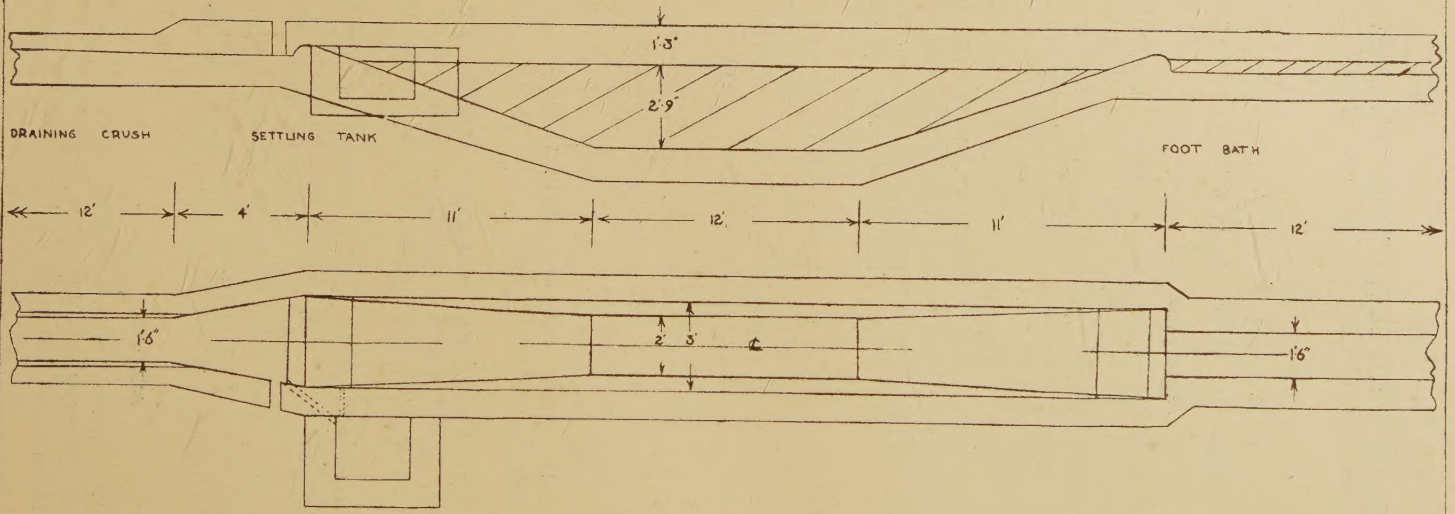
This treatment would destroy the ticks attached to the lower and less accessible parts of the beast and if combined with treatment of the ears and perineum would probably be more effective than simple hand dressing alone. We therefore designed an experiment to determine the effect of gammexane washes on cattle, when applied in such a half-bath, by careful observation of the tick infestations.

METHODS

In order to simulate stock route conditions at their worst, it was necessary to expose the experimental cattle to heavy tick infestation after each treatment with acaricide and thus an area with a

EXPERIMENTAL HALF-BATH FOR CATTLE.

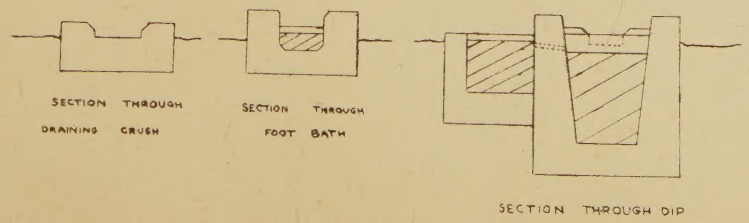
BUILT AT MLALI YA PILI IN THE CENTRAL PROVINCE OF TANGANYIKA



DIP BUILT OF DRESSED STONE AND CONCRETE. NO DETAILS OF THATCHED ROOF CONSTRUCTION OR OF CRUSH AND HOLDING PENS ARE INCLUDED.

THE TWO RAMPS ARE OF ROUGH UNFACED CONCRETE TO PREVENT THE CATTLE FROM SLIPPING.

CAPACITY OF BATH 920 GALLONS.



M.R.B.

FIG. 1.

high tick population and preferably where East Coast fever was enzootic, was needed for the experiment. Mlali, in the Central Province of Tanganyika, was chosen. According to Wilson (1948a) the predilection sites for various species of tick on the bovine are so well marked that if those favoured by East Coast fever vectors are kept free from ticks the chances of infection with the disease are small. The sites selected for treatment were, therefore, the ears, the axillae, the inguinal region, the ventral parts of the abdomen and chest and the perineum.

The dipping tank was a trough 2 ft. 9 in. deep holding 920 gallons. It was made of concrete reinforced with roughly dressed stone and was covered with a thatched roof to minimise evaporation of the wash by the sun and dilution by rain water. The cattle walked through a shallow foot-bath and entered the dip by a ramp. On coming out, they stood in a short draining crush for a few minutes while the dip wash was poured over their heads and into their ears and applied to the perineum with a hessian swab. The drainings from the cattle were returned to the dip through a small settling-tank (Fig. 1). Before dipping was commenced, four special beasts were herded through the dip and back again to stir up the suspension. These animals were then dipped and the ears and perineum treated before the experimental herd passed through. No difficulty was encountered in driving the beasts through the wash, even though they had never been dipped before.

The product used for the preparation of the wash was "Gamatox 2," a paste which when made up at a dilution of 10 lb. to 1,000 gallons of water, produces a fine suspension of 50 parts per million of gammexane.

The ticks selected as indicators were the nymphs of all species and the adults of *Rhipicephalus appendiculatus, R. evertsi* and *Amblyomma variegatum*. Observations were continued for eight months and the wash was maintained by raising the liquid to the correct level with a suspension of the normal strength before each dipping. No attempt was made to test the strength of the dip during the experiment, but at the conclusion the gammexane content was found to be 64 parts per million.

DETAILS OF EXPERIMENT

The experiment started in the height of the dry season when the tick population was relatively small. In order to get a significant number of ticks for dipping, ten cows with exceptionally heavy tick infestations were selected from local herds. Most of the cows in this group had calves at foot and these were dipped together with their dams. There were not enough of the more heavily infested animals available at this stage to act as controls. Five more cows, however, were brought under observation as controls when the early dippings had reduced the tick infestation of the dipped cattle to a level approximating that of the control animals. It

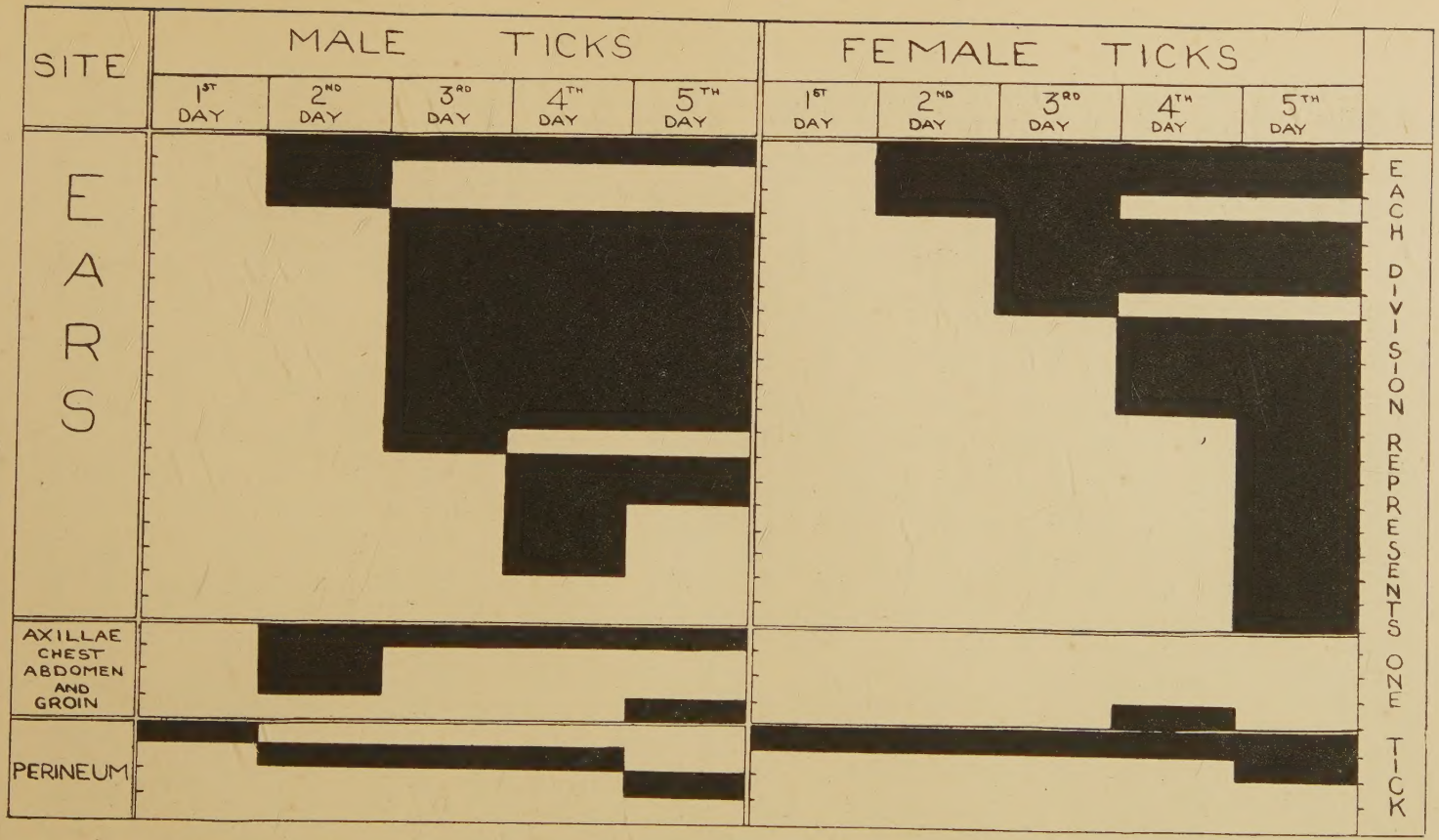


Fig. 2.

DIAGRAM SHOWING HISTORIES OF INDIVIDUAL TICKS ON THREE EXPERIMENTAL ANIMALS.

was considered that the small number of cattle used in the experiment could not appreciably affect the tick population of the common pasture and bush on which they were grazed. The cows were cast and the ticks in the selected sites were counted without being removed and distinction was made between species, nymphs, males, unengorged females and engorged females. During the first 22 weeks of the experiment the animals were dipped once weekly (immediately after the counting) and during the last ten weeks at intervals of five, five and four days, *i.e.*, three times per fortnight as suggested by Thorburn (1947). During the first and last fortnights of the experiment ticks were counted daily, but for the rest of the period counts were made once weekly. Between dippings, the cattle were grazed on the tick infested pastures.

During the last fortnight an attempt was made to follow the histories of individual ticks on three of the dipped animals. A chart on which locations of the ticks were marked was drawn for each animal. Each day the positions were noted. The results of this incidental investigation are tabulated in Fig. 2.

RESULTS

Throughout the experiment the distribution of tick species was found to follow fairly closely that described by Wilson (1948a). *R. appendiculatus* were found chiefly on the ears and occasionally on the belly, axillae and groins, *A. variegatum* were found on the belly, axillae and groins and occasionally on the perineum and *R. evertsi* were confined to the perineum. Occasional total tick counts on the animals showed that only a very small proportion were attached to parts other than those described as predilection sites. During the first two days after dipping, the total numbers of ticks fell steeply: thereafter, an increase occurred up to the seventh day, when dipping was repeated. A second steep fall and a subsequent gradual rise, although to a lower peak than on the previous week, then occurred (Fig. 3). After the first dipping, the numbers of nymphs fell from 406 to 16 on the first day and to eight on the second day after dipping. Attachment of fresh nymphs and adults had begun by the third day.

The best guide to the efficiency of a dip is the observation of engorged females. For example, the presence of engorged *R. appendiculatus* females on an animal indicates that the ticks must have been attached for at least 96 hours (Monning, 1947). Careful notes were, therefore, made of the engorged females found and these are recorded in Table I and also shown in the histogram accompanying Fig. 4. As it was often difficult to ascertain the exact state of engorgement without disturbing the ticks, females were recorded as engorged if they were more than half distended.

The distribution and total numbers of engorged female ticks of all the mentioned species are shown in Table II. The trend of the

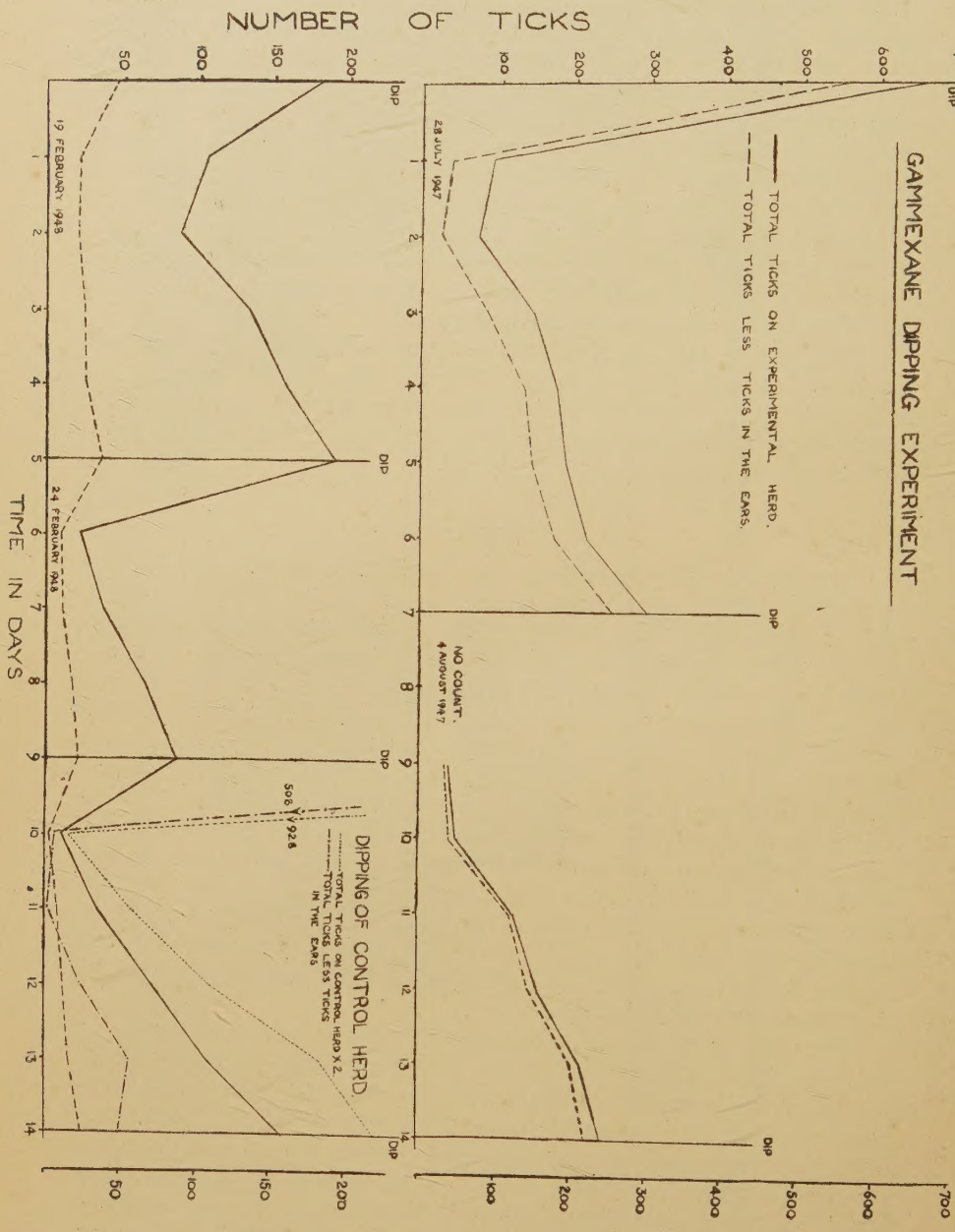

NUMBER OF TICKS
GAMMEXANE DIPPING EXPERIMENT
TOTAL TICKS ON EXPERIMENTAL HERD.
TOTAL TICKS LESS TICKS IN THE EARS.
28 JULY 1947
NO COUNT. 4 AUGUST 1947
DIPPING OF CONTROL HERD.
TOTAL TICKS ON CONTROL HERD X 2.
TOTAL TICKS LESS TICKS IN THE EARS
19 FEBRUARY 1948
24 FEBRUARY 1948
TIME IN DAYS
DIP

FIG. 3.

TABLE I

RECORD OF THE NUMBER OF ENGORGED FEMALE TICKS ON THE DIPPED HERD AND CONTROL HERD

Weekly dipping				*Dipping three times every fortnight*			
Date	*Number of times dipped*	*Experimental herd*	*Control herd (X2)*	*Date*	*Number of times dipped*	*Experimental herd*	*Control herd (X2)*
27.7.47	Before dipping	9					
3.8.48	1	26		2.1.48	23	3	12
10.8.47	2	0		7.1.48	24	2	6
17.8.47	3	30		12.1.48	25	0	2
24.8.47	4	19	16	16.1.48	26	0	14
1.9.47	5	10	20	21.1.48	27	0	No count
7.9.47	6	2	22	26.1.48	28	0	30
14.9.47	7	1	20	30.1.48	29	0	32
21.9.47	8	5	32	4.2.48	30	1	54
28.9.47	9	8	42	9.2.48	31	0	58
5.10.47	10	6	26	13.2.48	32	1	38
12.10.47	11	4	2	18.2.48	33	5	38
19.10.47	12	2	8	23.2.48	34	0	20
26.10.47	13	0	26	27.2.48	35	0	26
2.11.47	14	0	4	3.3.48	36	0	
9.11.47	15	0	6				
16.11.47	16	4	6				
23.11.47	17	2	12				
30.11.47	18	9	24				
7.12.47	19	3	58				
14.12.47	20	10	50				
21.12.47	21	1	6				
28.12.47	22	2	14				

TABLE II

DISTRIBUTION OF ENGORGED FEMALE TICKS

Site	*Experimental herd*		*Control herd*	
	Number of engorged females	*% of total engorged females*	*Number of engorged females*	*% of total engorged females*
Ears	8	8	20	2·75
Axillae	12	12	104	14·4
Groin	7	7	38	5·25
Chest and abdomen ...	63	63	536	74
Perineum	10	10	26	3·6
Total	100		724	

The total number of engorged females of all three species and their distribution from the fourth dipping to the end of the experiment,

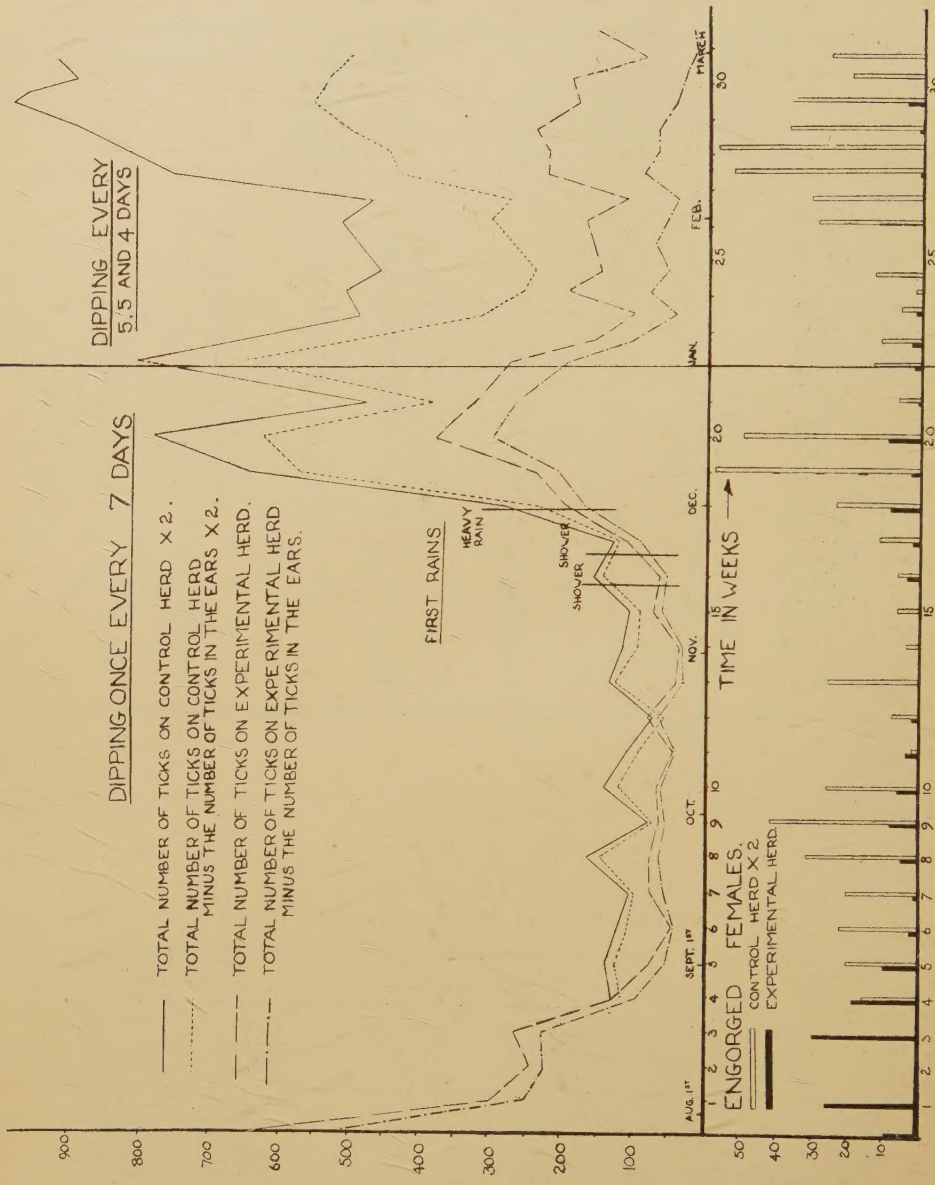

DIPPING ONCE EVERY 7 DAYS
TOTAL NUMBER OF TICKS ON CONTROL HERD X 2.
TOTAL NUMBER OF TICKS ON CONTROL HERD MINUS THE NUMBER OF TICKS IN THE EARS X 2.
TOTAL NUMBER OF TICKS ON EXPERIMENTAL HERD.
TOTAL NUMBER OF TICKS ON EXPERIMENTAL HERD MINUS THE NUMBER OF TICKS IN THE EARS.
DIPPING EVERY 5,5 AND 4 DAYS
FIRST RAINS
HEAVY RAIN
SHOWER
SHOWER
AUG. 1ST
SEPT. 1ST
OCT.
NOV.
DEC.
JAN.
FEB.
MARCH
TIME IN WEEKS
ENGORGED FEMALES.
CONTROL HERD X 2.
EXPERIMENTAL HERD.

FIG. 4.

total tick population on the experimental animals compared with that on the control animals is shown by Fig. 4. In addition, the chart contains the graph of total ticks less the ticks in the ears, for both experimental and control animals. During the first half of the experiment, the tick numbers remained at a fairly low level in all the animals. With the onset of the rains the population on both experimental and control animals rose, but there was a marked divergence of the two curves; that for the control animals rising very steeply. At the end of December when the rains were well established these curves appeared to have attained their peak levels, and dipping at intervals of five, five and four days was substituted for weekly dipping. This caused a marked reduction of the tick population, the curve falling once more. The curve of the control animals, however, continued to rise.

The effect of dipping at intervals of five, four and five days respectively is shown in Fig. 3 and Fig. 4 in which are plotted the total tick numbers on the experimental animals together with the totals less the numbers in ears. At the final dipping the control animals were also passed through the bath. The effect of this immersion is shown in Fig 3. The total number of ticks before dipping was 1,928; one day after dipping, there were 14 of which eight were in the ears; five days after dipping the number had risen to 222, of which 172 were in the ears. Comparison of the number of ticks in the ears expressed as a percentage of total ticks, with corresponding figures for the control animals, is demonstrated in Table III.

At the beginning of the experiment there were twelve calves which were dipped regularly. Three of these must have been infected before dipping was commenced, since they died of East Coast fever in the first fortnight. None of the remainder, including three which were born to experimental animals during the experiment, died and none showed signs of East Coast fever, although in this area the inhabitants expected to lose more than half their young cattle from this disease within about four months from birth.

DISCUSSION

Gammexane in the form of the proprietary dip "Gamatox 2," used as a suspension of approximately 50 parts per million in water kills nymphs and adults of *R. appendiculatus*, *R. evertsi* and *Amblyomma variegatum* on cattle when these are treated in the manner described above.

In this experiment, weekly dipping reduced the level of the tick population on the treated cattle. No repellent action is claimed for gammexane (Thorburn, 1947) so that the attachment of ticks three days after dipping does not necessarily indicate the termination of the residual action of the preparation: it is possible that ticks attaching so soon after dipping may not be able to engorge. In an experi-

ment staged under the conditions described here, satisfactory observation of the effect on the individual tick, with regard to its attachment to the dipped animal, was not possible. Nevertheless, the small experiment represented graphically in Fig. 2 indicated that a few ticks were capable of attaching themselves to cattle on the second day after dipping. One female appeared on the first day after dipping, but neither this nor any of the other ticks engorged.

TABLE III

NUMBER OF TICKS IN THE EARS EXPRESSED AS A PERCENTAGE OF THE TOTAL TICKS

		Experimental herd			*Control herd* (X 2)		
Date	*Number of times dipped*	*Total ticks*	*Ticks in ears*	*% Ticks in ears*	*% Ticks in ears*	*Ticks in ears*	*Total ticks*
27.7.47		649	114	17·5			
3.8.47	1	301	48	16			
10.8.47	2	245	19	7·8			
17.8.47	3	268	40	15			
24.8.47	4	123	24	19·5	11	14	126
1.9.47	5	78	23	29·5	9	12	134
7.9.47	6	49	8	16	11·5	14	120
14.9.47	7	75	17	22·5	9·25	10	108
21.9.47	8	80	16	20	11	18	162
28.9.47	9	67	9	13·5	7·5	6	80
5.10.47	10	71	7	10	14·3	20	140
12.10.47	11	49	5	10	16·5	18	110
19.10.47	12	82	13	16	19·5	14	72
26.10.47	13	46	14	30	7·35	10	136
2.11.47	14	38	6	15·8	15·7	18	114
9.11.47	15	16	14	18·5	13·2	14	106
16.11.47	16	69	10	14·5	8·9	14	158
23.11.47	17	117	18	15·4	9·2	12	130
30.11.47	18	119	29	14·5	15	42	280
7.12.47	19	208	56	21	11·4	74	648
14.12.47	20	383	83	21·5	19·2	150	780
21.12.47	21	326	56	17	18·5	88	472
28.12.47	22	284	77	27	18·3	138	754
		Rate of dipping: Weekly					
2.1.48	23	170	63	37	28	192	686
7.1.48	24	107	62	58	35	172	490
12.1.48	25	196	115	58·6	49	250	510
16.1.48	26	150	91	60·5	47	214	458
21.1.48	27	163	87	53	—	—	—
26.1.48	28	171	110	64	42	216	518
30.1.48	29	111	67	60·5	41	192	472
4.2.48	30	225	134	59·5	42·5	322	760
9.2.48	31	227	154	68	48	396	830
13.2.48	32	244	170	70	43	374	878
18.2.48	33	182	136	71	44	434	990
23.2.48	34	192	158	82	40	360	896
27.2.48	35	87	66	76	45	420	928
		Rate of dipping: Every 5, 5 and 4 days					

Thirteen of the ticks attaching after dipping, failed to remain on the animals until the fifth day. This finding, although not conclusive, is suggestive of a residual action of the gammexane.

During the experiment the tick counts never fell to zero and the fact that a few ticks can remain attached for four or more days between dippings presents the possibility of full engorgement and the transmission of the sporozoites of *Theileria parva*. It must be noted, however, that in this experiment (with the exception of the first and last fortnights) tick counting was always done immediately before dipping, so that seven days had elapsed since the previous dipping during which period the cattle were exposed to infestation. The curve for the experimental animals in Fig. 4 therefore represents the numbers of ticks which had attached to the animals during seven days subsequent to treatment with gammexane. The actual killing effect of the wash is shown graphically in Fig. 3.

In the majority of counts the proportion of the ticks in the ears to the numbers on the rest of the body was high when compared with the corresponding ratio in the control animals (Table III). This was probably due to the greasiness of the inside of the ears which prevented the spread of the wash and consequent deposition of active acaricide. The method of application to the ears was therefore unsatisfactory.

According to Wilson (1948a), if the predilection sites of the ticks are kept free, the chance of infection with *Th. parva* is greatly reduced. We contend that the infective ticks in their predilection sites are killed when these sites are treated with the acaricide. Those attaching after treatment are adversely affected by the residual acaricide, or are killed by the next application. Ticks feeding on untreated parts will in most cases have passed over treated areas before reaching their feeding places and, depending on the degree of residual action, will be correspondingly poisoned. Furthermore, recent work by Wilson (1948b) suggests that under conditions which favour an enzootic, infection by a small number of ticks may produce only mild symptoms of East Coast fever, followed by immunity in animals so affected. It is thus possible that the correct application of gammexane will prove an important method of controlling mortality from East Coast fever.

Criticisms have been made that the insecticidal properties of gammexane dip washes when used in plunge baths decrease considerably after a few months. No such decrease was recorded in this experiment as is shown by the effect of the dip on both the experimental and control animals after it had been in use for over seven months (Fig. 3). This was probably due to two factors: (a) the bath was shallow, and as the animals walked through it their feet stirred up the sediment and caused more efficient mixing than occurs when they swim through a plunge bath; (b) the capacity

of the bath was so small—920 gallons—that the fresh wash added at intervals formed a relatively larger proportion of the total volume than it would be in a large tank of 4,000 or 5,000 gallons.

The change from weekly dipping to dipping at intervals of five, five and four days resulted in a significant fall in the tick population on the experimental cattle at a time when the seasonal increase was most marked in the control animals. During this period of dipping at shorter intervals, no engorged females were found on the dipped animals, whereas large numbers were present on the controls (Fig. 4). This indicates that dipping at these intervals is a possible means of control of East Coast fever. Practical evidence in support of this opinion is given by the apparent protection afforded to the calves by their passing through the bath with the experimental animals.

We consider that the results obtained in this experiment justify the trial of gammexane half-baths on stock routes. Combined with hand dressing of the ears, such half-baths should give greater protection against ticks than does hand dressing alone. It is suggested that as building materials are costly and where water is scarce, gammexane half-baths could satisfactorily replace full size arsenical plunge-baths with the added advantage of eliminating the dangers of arsenic poisoning.

The half-bath described in this communication can be constructed more cheaply than a full sized plunge bath and it is more economical to fill and maintain. This is an important consideration in arid and semi-arid areas. Furthermore, whereas some users of gammexane wash in plunge baths have complained of a rapid falling off of its efficiency after a few months, its acaricidal value appears to be maintained in a half-bath, possibly for the reasons described above. We are of the opinion, however, that for routine use a stronger wash than was used in the experiment is neccessary and suggest that a gamma isomer concentration of 75 parts per million be taken as the minimum strength in East Coast fever enzootic areas. In addition, we think that the effective depth of the dip should be 3 ft. 6 in. and not 2 ft. 9 in.

The method employed for the application of the wash to the ears was inefficient and also cumbersome and wasteful. Special attention should be paid to the treatment of these sites. Hand dressing with an oily preparation in a long draining crush after dipping would seem to be preferable, or, if the gammexane wash is used, it should be applied by sprays capable of emitting the wash under high pressure in order to overcome the greasiness of the ears.

SUMMARY

The construction of a walk-through half-bath for cattle, built in the Central Provinces of Tanganyika Territory for use with the acaricide "Gamatox 2," is described.

Over a period of seven months a herd of ten cows was dipped

in a suspension containing approximately 50 parts per million of gamma benzene hexachloride. The interval between dipping was at first seven days but after the start of the rainy season this was decreased to intervals of five, four and five days. Ticks were counted immediately before each dipping. The tick infestation of five undipped beasts used as controls is recorded.

The areas on the beasts chosen for the detailed observation of the acaridical properties of gammexane were the ears, axillae, abdomen and chest, inguinal region and the perineum. *Rhipicephalus appendiculatus*, *R. evertsi* and *Amblyomma variegatum* were used as indicators.

The significance of the results obtained by the treatment of these predilection sites of ticks on the control of East Coast fever is discussed.

Practical suggestions for the design of half-baths and the use of gammexane washes are made.

ACKNOWLEDGMENT

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A DISEASE OF ICELANDIC CATTLE CHARACTERISED BY SUDDEN DEATH

By

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INTRODUCTION

In recent years a number of cows in Iceland have been lost through a disease of unknown etiology. Farmers call the condition "Brádadaudi" which means sudden death. The disease has probably occurred in Iceland for a long time, but as it has been especially conspicuous during the last eight or ten years, it is believed that it has become much more prevalent than before. The geographical distribution of the cases is erratic, as deaths may occur simultaneously in widely separated districts without any apparent connection between them, and in each area the incidence varies greatly from year to year. On most farms not more than one or two cows are struck each year, but heavier losses are not uncommon. Last winter one farmer lost eight cows out of a herd of 15 which is rather larger than the average size of a single herd in Iceland.

CLINICAL FEATURES

Because of the suddenness of death which characterises the disease, we have never had an opportunity to observe a living case. The following information which has been obtained by questioning farmers is believed to be reliable. It is usually asserted that the cows died suddenly and without any obvious premonitory symptoms. We have collected information on 134 fatal cases. Some of the

Table I

Analyses of Death in 312 Cows

Died in less than 15 *minutes*	*Final illness lasted more than* 15 *minutes*	*Symptoms of same disease had appeared earlier or the final illness lasted more than* 24 *hours*	*No earlier signs of the disease. Duration of illness unknown although always less than eight hours*	*Died in the stable*	*Died at pasture*	*Known connection with physical strain*	*Died at milking time or less than one hour afterwards*
49	6	36	43	72	62	30	14
36·5 %	4·5 %	26·9 %	32·1 %	53·7 %	46·3 %	22·4 %	10·4 %

results are recorded in Table I, which gives certain general information regarding the behaviour of the disease. It was found that 49 cows died in less than 15 minutes after becoming sick without having previously shown any symptoms of disease: many were said to have died almost instantaneously. Six lived for more than 15 minutes but had never before shown symptoms of the disease. Thirty-six cases were in animals which had apparently recovered from an attack and had suffered a relapse or else in which the initial attack lasted more than 24 hours. The remaining 43 animals were found dead under circumstances in which it was not possible to determine exactly how long they had lived after the onset of illness, but they had not shown symptoms previously. In these cases also the illness probably lasted for a very short period only. Seventy two animals died while at pasture and 62 died in the stable. In 30 cases some definite connection with severe physical strain was noticed and in 14 cases death occurred at or shortly after milking time. Table II,

Table II

Seasonal Incidence of Cases of "Brádadaudi" (135 cases)

Jan.	*Feb.*	*Mar.*	*Apr.*	*May*	*June*	*July*	*Aug.*	*Sept.*	*Oct.*	*Nov.*	*Dec.*
1	8	14	10	26	35	17	9	7	0	2	6

based on 135 fatal cases, shows the seasonal distribution of the deaths. The largest number occurred during the period March to July and they were most frequent during the month of June. This information is based on data gathered from 50 farms during the years 1943 to 1948.

The clinical appearance of the cases was usually as follows. The cow which was in perfectly good health and well nourished usually fell down without any premonitory symptoms whatever, groaned for a few minutes and then died. No convulsions were noticed. In a minority of cases, the animal had been dull and listless for several days before it died. In other cases these premonitary symptoms developed earlier, but instead of ending in death the animal apparently made a complete recovery but symptoms recurred later, occasionally several times at varying intervals, and death finally supervened. The excellent health of most of the cows until the very moment of the dramatic attack should be emphasised and the apparently complete but temporary recovery from an attack is also worthy of particular attention.

The observations reported above agree in general with the previous findings of Gunnarsson (1945). He found, however, an even more pronounced seasonal variation in the deaths and also reported an apparent tendency of foodstuffs of high value, such as cornmeal, herring meal, etc., to reduce the incidence of "Brádadaudi" to some extent.

ANATOMICAL FINDINGS

We have had the opportunity to perform autopsies on a number of cases of the disease and have also examined organs from other cases. All the carcases were from animals which had obviously been in good condition and no gross macroscopic lesions were seen. In five cases, small petechial haemorrhages were found under the epicardium. The voluntary muscles were examined carefully and their colour and consistency were found to be normal. The brains were removed in three cases, but no visible lesions were found.

The microscopic tissue changes in this disease will not be discussed at length, as our histological investigations are not as yet sufficiently advanced. It may be mentioned, however, that rather characteristic lesions of the kidneys were observed. Bowman's capsule contained considerable detritus staining faintly with eosine. The same material was found to a varying degree in the tubules, particularly the convoluted tubules. In some cases this detritus filled the capsule to such an extent that the glomerular tuft was displaced towards the wall so that some sections would not reveal any of the tuft but only the detritus. In other cases only a small part of the tuft was seen, the detritus filling most of the space (Fig. 1). It is believed that at the time of fixation there was fluid in the nephron which contained a protein that was coagulated by the fixing solution and that the detritus seen in the sections is the precipitate from the coagulation.

In the renal pyramids recent haemorrhages which infiltrated the tissue were always found (Fig. 2). These haemorrhages which were small in size were always multiple and even numerous. Interspersed with the fresh haemorrhages were remains of older ones in the form of pigment (haemosiderin) granules.

AETIOLOGY

The increased prevalence of the disease has stimulated efforts to elucidate its aetiology. The first attempt in this direction was made by Professor Jón Steffensen of the University of Iceland who determined the calcium, magnesium, and inorganic phosphorus content of the blood of approximately 45 cows monthly for a period of one year (October, 1944 to September 1945, results unpublished); 33 of these cows were from farms where "Brádadaudi" was prevalent and twelve were from farms where the disease had never occurred. He also determined the mineral content of sera taken immediately after death from twelve cases of "Brádadaudi." In every case the calcium and magnesium contents were normal. On the other hand in his general survey of healthy cows from the affected farms, he found an unexpected seasonal variation in the inorganic serum phosphorus whilst the figure for the "Brádadaudi" cases was also definitely outside the normal limits. It was concluded, that hypomagnesaemia or hypocalcaemia was not the cause of these deaths

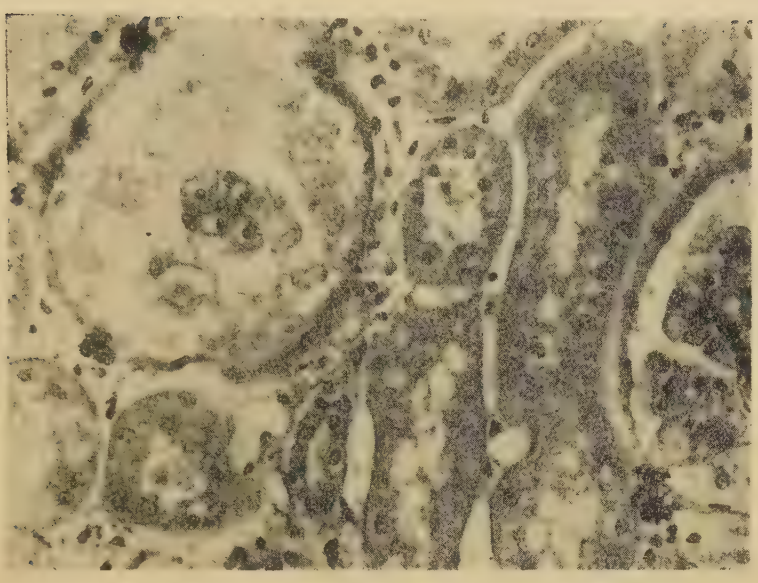

FIG. 1.—A section from the renal cortex of a brádadaudi case. Magnified 360 ×. A glomerulus is seen to be almost filled with detritus

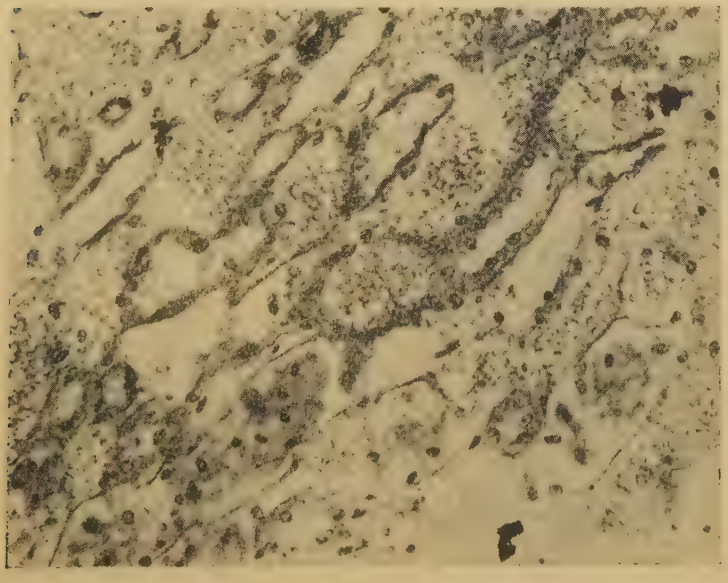

FIG. 2.—A section from the renal pyramid of a case of brádadaudi showing haemorrhage. Magnified 320 ×.

and that the disease in question was not identical with so-called grass tetany. (Sjollemma, 1932, Russell, 1944).

The next attempt to elucidate the nature of this condition was made in the winter of 1945 to 1946. One hundred cows on affected farms in the vicinity of Reykjavík were given a daily dose of synthetic vitamins and an equal number were kept on the same farms as controls. The composition of the supplement was as follows: Ascorbic Acid 200 mg., Nicotinic Acid 200 mg., Panthothenic Acid 0·5 mg., Pyridoxin 0·5 mg., Riboflavin 10 mg., Thiamin 10 mg. The cows received this dose for approximately 200 days. The results were entirely negative since five of the animals from the experimental group and one from the control group died of "Brádadaudi." It is well known that animals deficient in riboflavin may die suddenly (Wolbach and Bessey, 1942), while in man a deficiency of ascorbic acid and other water soluble vitamins may cause a sudden collapse. This was our reason for trying these vitamins although the microflora of the intestines of ruminants is supposed to supply them in adequate quantities.

Australian workers have described a condition in cattle called Falling Disease or Sudden Death (Bennetts *et al.*, 1939, 1941, 1942) which from their description would be difficult to distinguish from "Brádadaudi." The clinical course—insofar as there is such a thing in this condition—seems to be similar. The seasonal variation in the incidence is the same in both conditions and the histological changes in the kidney are strikingly similar, although we do not think that the glomerular tufts are destroyed as these authors seem to believe, but rather that they have been pushed aside by the protein-containing fluid inside the nephron. This could conceivably happen at the moment when, due to oncoming death, the blood pressure falls. The capillary tuft would then collapse and some of the fluid in the tubules would be able to flow back into the Bowman's capsule. Myocardial fibrosis described by the Australian authors in most of their cases has not yet been observed in "Brádadaudi" and this seems to be the only discrepancy between their findings and ours.

The Australian disease occurs in districts which are deficient in copper and in animals where symptoms of copper deficiency of a varying degree are evident. The Australian authors therefore regard Falling Disease as the terminal stage of copper deficiency. This opinion is apparently based on a small controlled experiment and on the fact that the disease has disappeared from the areas where copper has been fed to relieve the deficiency. On the basis of these observations we started feeding a mixture of copper and cobalt in the fall of 1946. Between 80 and 90 cows received this supplement until the spring of 1948 with the exception of a period in the late summer of 1947. The treatment thus lasted through two "Brádadaudi" seasons. A similar number of animals on the same farms were kept as controls. Each animal received a daily

TABLE III

INCIDENCE OF "BRÁDADAUDI" IN A GROUP OF COWS FED A SUPPLEMENT OF COPPER AND COBALT AND IN A CONTROL GROUP

	Animals fed copper and cobalt		*Control animals*	
	Total	*Died*	*Total*	*Died*
First period. Fall of 1946 until Dec. 31st, 1947 ...	89	4	88	5
Second period. Jan. 1st, 1948, until June 30th, 1948	79	4	80	1
Total	168	8	168	6

dose of 300 mg. of copper sulphate and 4 mg. of cobalt chloride: these additions to the normal feed did not seem to affect the general health of the cattle. The results of this experiment are shown in Table III from which it will be seen that the supplement of copper and cobalt did not prevent the occurrence of "Brádadaudi."

Organs from nine cases of "Brádadaudi" have been tested for copper. The results of the analyses are given in Table IV which shows that all the values fall within the normal range. Six samples of hay from "Brádadaudi" districts were tested for copper: the values calculated on a dry matter basis were 14·0; 10·4; 9·2; 6·4; 19·0 and 19·1 p.p.m. and fall within the normal range.

The mean value of blood copper for 25 samples of blood taken from cows on affected farms at the peak of the disease period was 0·094 mg. per cent., with a range of 0·08 mg. per cent. to 0·12 mg. per cent. Determination of the calcium and magnesium contents of sera from 25 cows on affected farms, showed the mean values to be 9·56 mg. per cent for calcium and 2·34 mg. per cent. for magnesium. A rather low magnesium concentration, *i.e.*, 1·6, 1·7 and 1·8 mg. per cent. respectively was found in three sera, but in general the findings of Steffensen were confirmed.

TABLE IV

COPPER CONTENT OF ORGANS FROM CASES OF "BRÁDADAUDI"

Cow number	*Heart*	*Liver*	*Lung*	*Kidney*	*Muscle*
4	18	254	—	20	—
5	18	202	19	25	65
6	16	138	8	15	7
7	18	98	7	16	—
8	16	156	12	18	—
9	19	156	9	17	—
10	19	574	11	21	—
11	—	220	8	14	—
12	14	156	8	20	—

The figures indicate p.p.m. on dry matter basis.

From the feeding experiment and the analytical results described above, we believe it may be safely concluded that " Brádadaudi " in Iceland is not due to a nutritional deficiency of copper. In further confirmation of this conclusion it should be mentioned that during the period from April to June, 1947, 266 blood examinations were made from 90 cows. Each animal was tested three times at an interval of about one month. The haemoglobin values and red cell counts were determined. The cows tested were from farms where copper and cobalt had been administered, half of the number being selected from the copper-cobalt group and the other half from the control group. It is evident from Table V that there was no differ-

TABLE V

COMPARATIVE BLOOD COUNTS AND HAEMOGLOBIN DETERMINATIONS ON COWS THAT HAD RECEIVED A SUPPLEMENT OF COPPER AND COBALT AND CONTROL ANIMALS FROM THE SAME HERD

	No. of cows	*Hb. per 100 ml.*	*No. ery-thoc.*	*No. leu-cocyt.*	*M.C.H.*	*No. of cows*	*Hb. per 100 ml.*	*No. ery-thoc.*	*No. leu-cocyt.*	*M.C.H.*
			Mil-lions	*Thou-sands*				*Mil-lions*	*Thou-sands*	
April 28th to May 22nd	45	11·2	5·2	6·3	22·3	44	11·3	5·2	6·4	23·3
May 29th to June 9th	45	11·7	5·0	5·9	24·3	47	11·9	4·7	6·0	25·5
June 27th to July 7th	43	12·3	4·5	6·3	27·2	42	12·1	4·5	5·9	27·1

M.C.H. indicates mean corpuscular haemoglobin.

ence between the copper-cobalt group on the one hand and the control group on the other as regards haemoglobin content and number of blood cells. During the period of observations, however, the mean haemoglobin content rose considerably in both groups, while there was a concomitant fall in the number of red blood cells. Consequently there was a rise in the mean corpuscular haemoglobin value (MCH).

SUMMARY

" Brádadaudi," a disease of cows, occurs in many widely separated areas in Iceland and the incidence varies from year to year in an entirely unexplained fashion. Affected animals die suddenly often without previous clinical symptoms. The disease reaches its peak during the spring and early summer. Cases frequently occur during or shortly after severe physical strain. On *post-mortem* examination gross macroscopic lesions are usually absent and conspicuous microscopic lesions are found only in the kidneys. These comprise small haemorrhages and a considerable exudation inside the nephron.

The result of some feeding experiments and certain chemical analyses suggest that the disease is not caused by hypocalcaemia, hypomagnesaemia, by a deficiency in ascorbic acid, nicotinic acid, panthothenic acid, pyridoxin, riboflavin or thiamin, nor by a deficiency in copper or cobalt. Clinically " Brádadaudi " is very similar to Falling Disease or Sudden Death of cattle in Australia which is believed to be caused by a deficiency of copper.

The aetiology of the Icelandic disease has not yet been explained.

ACKNOWLEDGMENT

Dr. Ruth Allcroft, of the Ministry of Agriculture Laboratory, Weybridge, England, carried out the copper analyses mentioned in this paper and also the serum analyses for magnesium. We wish to express here our sincere thanks for this valuable assistance.

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BLUETONGUE OF SHEEP IN CYPRUS

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INTRODUCTION

An outbreak of a disease termed "stomatitis" was observed among sheep in Cyprus in November, 1924, by Roe (1924) who stated that no similar affection had been previously seen in the affected areas even by the oldest shepherds. The disease was characterised by high fever, catarrhal stomatitis with swelling of the lips and in some cases ulceration of their borders, salivation, nasal discharge and lameness or stiffness in one or more limbs. Only sheep were affected and no cases were seen in goats although the latter were running with the affected flocks and had every opportunity of becoming infected.

The disease was widespread until the following March, but seemed to disappear by the summer. It recurred on a wide scale in the autumn of 1926 and persisted until the following January. In December, 1927, the disease was reported from two village areas, and in the latter half of 1928 there was a further outbreak which was, however, less extensive than that of 1926 and in which most cases were of a benign nature. It was reported both in 1930 and 1936, but on each occasion it was confined to a single village area and affected only a handful of animals.

The disease appeared again in August, 1939, in a very widespread outbreak affecting nearly all the low-lying parts of the Island and persisted until the end of November. In the earlier months the affection was very mild and caused little loss, but during the colder weather many animals died. In individual flocks losses up to 10 per cent. were noted, but on the whole the mortality was very much lower and was estimated at less than two per cent. of the total sheep of the Island.

At that time investigations were very limited. Attempts were made to transmit the condition experimentally to healthy sheep by housing these in close contact with affected animals, by rubbing the gums and dental pad with saliva and necrotic material from mouth lesions, and by the subcutaneous injection of an emulsion of spleen pulp from a sheep which had died of the disease. None of these attempts were successful, although the sheep inoculated with spleen emulsion showed an elevation of temperature (maximum 105·4) from the seventh to ninth and the 12th to 16th days, without any other symptoms.

In October, 1943, the disease appeared again in a more virulent form than had been seen previously and continued until the begin-

ning of January, 1944. The outbreak was not particularly widespread, but in some of the areas affected—especially in the larger flocks—losses were catastrophic and deaths were between 60 and 70 per cent. As abundant clinical material was available, laboratory investigations were commenced immediately. It was quickly shown that the disease could be reproduced experimentally by the intravenous injection of unfiltered and filtered blood from infected animals but was not transmitted by simple contact or by the application of discharges. The similarity of the symptoms to those of Bluetongue was soon noticed and the conviction that the two conditions were identical increased as the investigations continued. Samples of virus were forwarded to the Onderstepoort Laboratories, South Africa, for cross-immunity tests with known Bluetongue virus and the two conditions proved to be the same.

In October, 1946, another outbreak of Bluetongue commenced, starting from two widely separated foci at opposite corners of the Island, one in the S.E. of Famagusta District, which had been the starting place of the 1943 epidemic, and the other in the N.W. corner of Kyrenia District. The disease was mild to begin with, and was not reported until it had occurred in a number of village areas and was beginning to cause more severe symptoms.

It was reported by the Onderstepoort Laboratories that the Cyprus virus of 1943-44 was more virulent than any strain previously examined at Onderstepoort. This finding was of considerable interest, since Bluetongue had not until then been recognised outside Africa ; moreover, cases were mainly confined to the South and Central parts of the Continent. In the West it had been recorded as far north as French Sudan (Curasson, 1936), and in the East as far as Abyssinia (Tarantino, 1940). Piot Bey (1909) recorded an outbreak of disease in the Nile Delta, which he considered to be Bluetongue, but since it affected goats as well as sheep and lesions of the feet and alimentary tract were totally absent, it seems doubtful whether his diagnosis was correct. As the only characteristic lesion he mentioned was cyanosis of the tongue, Bluetongue, if present at all, must have occurred in a mild form probably complicated by the presence of some other infection, such as Contagious Pustular Dermatitis.

At the time of the 1943 outbreak, the same disease was seen in Palestine, although not in such a severe form (Simmins, 1944 ; Binns, 1944). Binns states that a number of years previously Gilbert had suspected the existence of Bluetongue in Palestine, but that the diagnosis had not been confirmed although material had been sent to Onderstepoort. In 1945 the Turkish Ministry of Agriculture reported Bluetongue among sheep. It occurred again in 1946 and 1947 at about the same time as the outbreaks in Cyprus. Aral (1948) states that the disease was first seen in Turkey in the autumn of 1944 when it caused very heavy losses in the affected flocks (total

deaths over 2,700). In subsequent outbreaks losses were on a much smaller scale. It was believed that the disease was introduced from Syria, where cases were said to be occurring at the time.

SYMPTOMS

The disease makes its appearance a few weeks after the onset of winter rains following a long dry summer period and dies out shortly after the beginning of the really cold weather. The first symptom is usually an elevation of temperature, although severe and even fatal cases are seen which are completely non-febrile. In experimental cases the incubation period varies from two to seven days, but three to four days is the rule. The maximum temperature is usually between 105° F. and 106° F., although temperatures above and below this range are frequent. The onset of the febrile reaction is followed, usually a day later, by nasal discharge and salivation. The nasal discharge is at first watery, but rapidly thickens and becomes mucous and is sometimes bloodstained. The nasal mucosa is often intensely congested and may show a purplish discoloration. There is sometimes excoriation of the epithelium and frequently deeper ulceration. The discharge can be very profuse and thick strings of mucus may be seen hanging from the nostrils. The salivation is sometimes extensive and is frothy rather than stringy. The lips and tongue are often much swollen and the margins of the lips bleed readily, especially when handled. The lips, gums and tongue are frequently congested and may show a purplish pink discoloration. Excoriation, or more often deeper ulceration, is sometimes seen on the lips. The tongue is occasionally a dark purple, but as in the majority of Cyprus sheep it is pigmented with black, these colour changes are commonly masked. The mucosa is frequently excoriated, especially at the tip or at the sides of the free portion and sometimes on the upper surface. The under surface may also be affected, but here a deeper ulceration, which sometimes becomes necrotic, is more frequent. These necrotic ulcers are usually regular in shape, elongated and lenticular. The most frequent site is at the sides of the fixed part of the tongue opposite the cheek teeth, but in most cases this can only be seen properly *post-mortem*. These ulcers are irregular in shape and distribution, corresponding with irregularities in the teeth. The inner surface of the cheeks is frequently congested and may show a superficial necrosis opposite uneven or sharp-pointed teeth. This also is seen more easily *post-mortem* than during life. Lesions on the upper jaw are not common, but dirty-greyish, firmly adherent deposits are sometimes seen on the dental pad. During the recovery stage scabs frequently occur round the lips.

The respiration is often stertorous, depending on the extent of nasal discharge, and animals may choke in their own secretions. Respiration is usually normal but may rise to 100 a minute. Symptoms

of pneumonia are common. The animal may cease to feed, or may eat very little. Thirst is often evident, though many animals will stand with the mouth immersed in water merely to ease the discomfort of the lesions and without making any attempt to drink. General digestive disturbances are frequent and diarrhoea, which is sometimes blood-stained, may occur. Blood may also be passed in the faeces in the absence of diarrhoea.

A purplish discoloration of the skin in the interdigital cleft is often noticed. Occasionally in the early stages of the disease the pasterns and coronets are swollen and may or may not be painful. An intense reddening of the skin just above the coronet has been seen in a few cases, but the distinct red band moving down the hoof with the growth of the horn during the recovery stage as described from South Africa (Thomas and Neitz, 1947) has not been noted in Cyprus. A purplish discoloration of the skin may also be seen in other parts of the body, under the sternum (where it is frequently accompanied by mild oedema), in the axillae, sometimes over the shoulders ribs and flanks, inside the thighs, occasionally on the udder and the flat under-surface of the tail (all Cyprus sheep are of the fat-tailed breed) and round the vulva and anus. The vulva and vagina are sometimes deeply congested.

If death follows it is usually from one to six days after the appearance of visible symptoms, but a few cases may linger on much longer. Some apparently make complete recoveries but subsequently collapse and die three weeks or more after the onset of the first symptoms. Many animals adopt an attitude of torticollis with the head bent round towards one flank. Recovered animals are usually in very poor condition and remain emaciated and debilitated for a long period. Sometimes during the illness and in the early stages of recovery many animals walk stiffly. It has been shown that this stiffness and the emaciation are due to a degeneration of the muscle fibres (Thomas and Neitz, 1947), but in Cyprus, owing to limited facilities, no histological studies were undertaken.

Prognosis is doubtful. Animals which seem but slightly affected, or those which appear to have recovered completely, may suddenly collapse and die, whereas animals which have shown severe symptoms for a considerable time may make complete recoveries. It is, however, a very unfavourable sign if the discharges from the nose or mouth are stinking, or if diarrhoea sets in, especially when bloodstained. In the 1943-44 experiments described later, 69 animals were artificially infected, of which 36 died. Nine animals developed stinking discharges and eight of these died. In the survivor, the unpleasant odour had been only slightly perceptible. Eighteen developed diarrhoea and 13 of these died, including four which passed blood. Two animals which passed blood without diarrhoea also died. In the five survivors the diarrhoea was slight. In the 13 which died, the diarrhoea would have been called slight in two cases only and in one of these blood was present in the faeces.

These symptoms in general closely resemble those described in Bluetongue by various S. African workers (Spreull, 1905 ; Theiler, 1906 : Bekker *et al.,* 1934), and in some of the standard text-books.

In the 1946 outbreak, the symptoms were similar to those seen in 1943, but stiffness of the legs was rather more in evidence in some areas, especially those where the disease was mildest. In some villages, many cases were seen where this was the only symptom and where but for the occurrence of a few typical cases in the same flock the reason for this stiffness might have been overlooked.

Post-mortem LESIONS

The lesions found in the mouth and nostrils at *post-mortem* examination, and also the discoloration of the skin, have already been described. Where the mouth lesions are pronounced, especially when they are necrotic, there is usually enlargement and congestion of the submaxillary lymph-glands. The lesions of the mouth and nostrils are naturally most pronounced in animals dying early in the course of the disease : where death occurs later than ten to twelve days after inoculation the lesions have usually healed. Apart from the above, there is little that can be regarded as in any way specific, the lesions being those of general disturbances of the respiratory, circulatory or alimentary systems caused by secondary infections in an animal already debilitated by the Bluetongue virus. The only description that will be given, therefore, will be a brief summary of the *post-mortem* findings in 36 experimental cases of the disease.

The glottis showed various degrees of congestion, usually accompanied by purplish discoloration, in seven and in two others there was oedema. The larynx was similarly congested in three and the trachea in ten. In 21 the lungs showed some degree of congestion or pneumonia ; only in one of them was pneumonia complete in both lungs while in another the lower half of each lung was solid. A predilection site for pathological change appeared to be the precardial lobe of the right lung which in three was completely solid where the rest of the lungs were healthy or only mildly congested, and in a fourth was intensely congested while the rest of the lung was only slightly affected. In three there were adhesions between the lungs and the pleura, a frequent finding in many natural cases of the disease. In ten the thoracic cavity contained fluid which was blood-stained in six. The pericardium contained fluid in eleven, seven of them blood-stained, including the six which had blood in the thoracic effusion. In 16 the heart showed small petechiae, 13 on the epicardium and eight on the endocardium ; otherwise the heart was usually normal, but occasionally the myocardium was a little dark in colour, sometimes a little flabby and in three congested. In one it was oedematous. Fluid was present in the abdominal cavity in eight, five times blood-stained, twice clear and once slightly purulent (in this case there was an old abscess in the wall of the rectum). The liver was congested in five, in addition to a number of chronic abnormalities such as hydatids, cirrhosis, focal necrosis, etc., which need not be detailed. The gall-bladder was distended in 17 and its lining congested in six. The spleen was soft or pulpy in 19 and enlarged without softening in one. The kidneys were congested in twelve with petechiae once and oedema once. In eleven the mucosa

of the rumen was congested usually over the pillars, but once along the course of the main blood-vessels, and once over the papillae: a twelfth showed congestion of the peritoneal surface. The reticulum showed congestion twice and the omasum once. The mucosa of the abomasum was congested or inflamed in 22 in one of which there were purple petechiae. In another case there was an ulcer close to the pylorus which showed considerable submucous haemorrhage at the sphincter in two cases, and congestion of the mucosa in two more. In one of the latter there was also haemorrhage into the muscles of the sphincter. Bekker *et al.* (1934) have called special attention to this lesion which seems to be of common occurrence in Bluetongue. In seven the duodenum showed abnormalities being either congested, containing blood, or being stained with bile. The jejunum and ileum were affected in 25 varying from slight congestion to acute inflammation. The caecum was congested or inflamed in 19, the colon in 18 and the rectum in four. In five the mucosa of the urinary bladder showed petechiae or congestion.

Small haemorrhages were occasionally noticed under the skin and in the skeletal muscles, but these were not nearly so frequent or so extensive as those described from S. Africa (Thomas and Neitz, 1947); nor was the gelatinous infiltration of the subcutaneous and intermuscular tissues noticed, although the deeper layers of the skin were often mildly oedematous, especially over the sternum.

Bacteriological investigations. No organisms were visible in smears from freshly dead cases of the disease. Cultures from the heart and spleen of slaughtered or freshly dead animals were consistently negative. In animals that had been dead for some hours, various organisms such as staphylococci, coliforms, and *Pseudomonas aeruginosa* were recovered, but these were obviously *post-mortem* contaminants.

EXPERIMENTAL WORK

Transmission Experiments

Attempts to transmit the disease to other species of animals. Rabbits were inoculated intravenously and guineapigs subcutaneously with whole blood from affected animals: none of these reacted. An ox was inoculated intravenously with blood from a sick sheep but failed to react. Later, a goat was inoculated with mixed blood from two experimental sheep, also without result.

Attempts to transmit the infection by discharges and by contact. The gums and tongue of an experimental Sheep (1) were well rubbed with a plug of cotton wool soaked with saliva and necrotic material from a mouth lesion in a naturally infected animal (Sheep A). Sheep 1 showed no deviation from normal health for a period of four weeks. This experiment was repeated later. Sheep 20, 21 and 22 were housed in a fly-proof shed, together with two severe natural cases of the disease (Sheep C and D). The mouth of Sheep 20 was well rubbed with saliva, necrotic material and nasal discharge from the two sick sheep: Sheep 21 and 22 were kept in contact. The same feeding and drinking vessels were shared by sick and healthy sheep. Sheep C died after six days while Sheep D made a slow recovery

and remained with the healthy sheep until the end of the experiment, which lasted a month. No reaction of any sort was seen in the experimental sheep.

Attempts to transmit the infection by inoculation. Sheep A was bled and 35 c.c. of citrated blood was injected intravenously into Sheep 2, which five days later began to show typical symptoms and died ten days after inoculation. On the seventh day 40 c.c. of citrated blood was inoculated intravenously into Sheep 3 and 2 c.c. of a Seitz E.K. filtrate into Sheep 4. Sheep 3 developed a febrile reaction accompanied by slight mouth lesions and nasal discharge, but eventually recovered. Eleven days after inoculation, blood was drawn and inoculated into Sheep 18 which died six days later, after showing more or less typical symptoms. Sheep 4, which had received the filtrate, showed a febrile reaction, profuse nasal discharge and torticollis. It collapsed eleven days after inoculation and was slaughtered *in extremis* on the twelfth day. Before slaughter, blood was drawn and 3·5 c.c. of a Seitz E.K. filtrate inoculated into Sheep 23, which failed to react—probably because the blood was drawn from Sheep 3 too late in the course of the reaction. Possibly sub-inoculation into another sheep might have produced a reaction (see discussion), but this was not known at the time.

Persistence of Virus

Experiments to test the persistence of virus in putrid and in preserved blood. Sheep 55 and 59 were inoculated intravenously with filtrates from a sample of blood from Sheep C and D, stored without preservative for three months. Both reacted mildly and recovered, but Sheep 66, inoculated intravenously with whole blood from No. 59, reacted fairly severely, though it finally recovered.

Blood from Sheep 39 preserved five weeks and seven weeks was inoculated subcutaneously into Sheep 56 and 62 respectively. No. 56 reacted only slightly, but 62 reacted severely and died. Sheep 65, inoculated intravenously with whole blood from No. 62, reacted moderately severely but recovered.

Preserved blood from Sheep 43 was used from one to two and a half years after collection. The reactions in the inoculated sheep were somewhat uncertain and erratic, but in many cases typical symptoms and deaths from Bluetongue followed in the susceptible animals used as controls and in vaccinated animals where immunisation had not been successful.

Protection Experiments

Studies on the curative properties of convalescent serum. In a seriously affected flock, blood was collected from convalescent animals. The serum, extracted and preserved by the addition of 0·5 per cent. phenol, was injected in 10 c.c. doses into animals in the early stages of the disease, some subcutaneously and some intravenously. The course of the disease was apparently not influenced.

Attempts to immunise with formolised vaccine. As soon as it became apparent that the causal agent of the disease was a filterable virus, attempts were made to produce a vaccine from formolised spleen pulp. The spleen was removed from an affected sheep immediately after death, ground up in a sterile mortar with ten times its weight of sterile saline and a little sterile sand and 0·75 per cent. formalin added. The sand and the coarser spleen particles were allowed to settle and the supernatant fluid was decanted off and bottled. Eight sheep (6 to 13) were vaccinated with this mixture in doses ranging from 0·5 c.c. to 4 c.c. and showed no unfavourable reaction. Two control sheep (14 and 15) were injected with 1 c.c. of formolised pulp similarly prepared from the spleen of a healthy sheep slaughtered for food. Twenty-six days later, all ten sheep each received 7·5 c.c. of whole blood from a naturally sick sheep. Five out of the eight vaccinated sheep and one of the controls died of the disease. In the meanwhile, as soon as the vaccine had been seen to be harmless, a field experiment was carried out in a badly affected flock. About a dozen sheep were inoculated with the spleen-pulp vaccine, but were not protected. Since both field and laboratory tests indicated that this was an unprofitable line of investigation, it was abandoned.

Attempts to attenuate the virus by passage. By this time it had appeared very probable that "Stomatitis" would prove to be the same as Bluetongue, so an attempt was made to attenuate the virus by serial passage through sheep after the method of Theiler (1908) and Du Toit (1929). Two strains from severely affected flocks were used, one from St. George's Monastery, Larnaca, and the other from the Cyprus Farming Co., at Kouklia. The strain of virus originally isolated from Larnaca (Sheep A) had been lost owing to Sheep 18 having died unexpectedly before blood had been collected for the next subinoculation. Sheep 19 had been inoculated at the same time with a mixture of bloods from Larnaca Sheep C and D. This strain of the virus was, therefore, used instead and maintained by regular subinoculation at approximately weekly intervals for 21 generations. Blood was also taken from Sheep 13, one of the animals inoculated with blood from a sick sheep from Kouklia, and this was maintained for 15 generations. The details of the inoculations involved and the results are shown in diagrammatic form (Fig. 1). The Kouklia strain appeared to become attenuated more rapidly than the Larnaca strain, but caused disappointment when it killed in the 13th and 14th generations: it then appeared to die out. This case, and the few other failures to react, may have been due to a natural or previously acquired immunity in some of the experimental sheep. The strain was carried on from a preserved sample of blood from the eleventh generation using as preservative the mixture of glycerol potassium oxalate and phenol described by Theiler and Du Toit. The 14th generation was again reached,

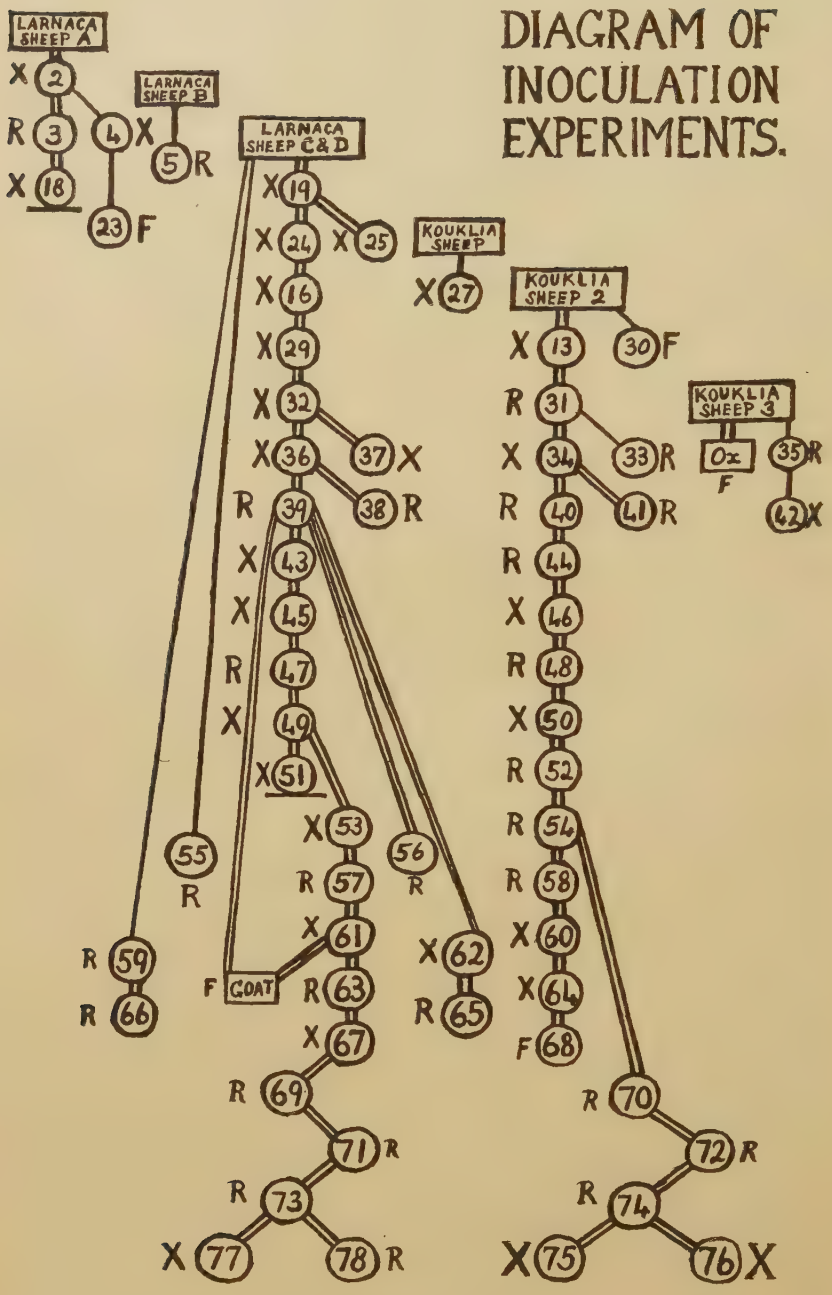
DIAGRAM OF INOCULATION EXPERIMENTS.
LARNACA SHEEP A
LARNACA SHEEP B
LARNACA SHEEP C & D
KOUKLIA SHEEP
KOUKLIA SHEEP 2
KOUKLIA SHEEP 3
Ox
F
GOAT

FIG. 1.

this time without further deaths and with quite mild reactions. In the 15th generation however, where two animals were inoculated, both died. The Larnaca strain at first showed no signs of attenuation, but later appeared to be gradually losing its virulence. From the 18th to the 20th generation there were no deaths and the symptoms shown were mild, but of the two animals inoculated in the 21st generation one reacted severely and died. No further attempts were made to attenuate the virus by passage. These results are discussed later.

VACCINATION OF SHEEP WITH MATERIAL RECEIVED FROM SOUTH AFRICA

Dr. R. A. Alexander, of Onderstepoort, very kindly supplied samples of three strains of vaccine for experimental purposes, *viz.*, Theiler's original "attenuated" strain and vaccines prepared from Bekker's and "Mimosa Park" strains respectively. He had found that the Mimosa Park strain gave complete immunity against Cyprus virus, that Bekker's strain gave less immunity and that Theiler's strain gave a very poor immunity and therefore would probably be unsuitable for field vaccination, especially in view of the possibility of its producing severe reactions. Details of the history of these three strains have been published (Neitz, 1948).

All three vaccines were used in a small-scale laboratory test during the winter of 1944-45. Three sheep were vaccinated with each strain and three used as controls. All three inoculated with Theiler's strain showed febrile reactions. Three weeks later, two of them died suddenly and unaccountably. Of the three inoculated with Bekker's strain, one showed a slight febrile reaction and 26 days later was found lying down in an attitude of torticollis, grinding its teeth violently: it died later the same day. The second sheep was destroyed following accidental injuries and *post-mortem* examination showed an enteritis which could not be satisfactorily explained. The third was not affected. The sheep receiving Mimosa Park vaccine showed no reaction except that one developed a considerable though transient salivation two days afterwards. Five

Explanation of Diagram

The circles containing numbers represent experimental sheep inoculated with Bluetongue virus, and rectangles the naturally infected cases from which the virus material was originally obtained or animals inoculated experimentally other than sheep.

Where circles or rectangles are connected by a double line, whole blood (fresh or preserved) was used for the inoculation. Where they are connected by a single line, inoculation was with a Seitz EK filtrate.

All filtrates were inoculated intravenously. In the case of inoculation with whole blood, this was intravenous where the circle is attached vertically downwards and subcutaneously where the attachment is oblique.

X = died; R = recovered from reaction; F = failed to react.

A horizontal line under a circle signifies that the animal died unexpectedly before blood could be collected for subinoculation.

weeks later the survivors and controls received an injection of supposedly virulent Bluetongue virus (preserved blood of sheep 43). The survivor of Theiler's strain showed fever and slight symptoms, and recovered. The survivor of Bekker's strain showed no reaction at all. All the animals vaccinated with Mimosa Park exhibited high fever and symptoms, but all survived. Only one of the controls developed typical Bluetongue and died after 15 days. Another showed mild fever with no symptoms, and the third failed to react.

In view of the good results obtained by Dr. Alexander with this strain, the Mimosa Park Test was repeated. Four sheep vaccinated with this strain were inoculated with virulent Bluetongue virus three to four weeks later, together with three controls. All the animals showed high fever and symptoms: two of the vaccinated and one of the controls died of Bluetongue.

Early in 1946 a supply of Cyprus virus (Larnaca strain) attenuated by serial passage through chick embryos was received from Dr. Alexander, and a laboratory test was made at once. Six sheep were vaccinated and showed no reaction to the vaccine ; six to nine weeks later these animals and four controls were inoculated with virulent Bluetongue virus. Three of the vaccinated animals showed no reaction to the virus and the other three showed high fever and slight symptoms from which they recovered completely, whereas two of the controls showed high fever and slight symptoms and the other two developed more or less typical attacks of Bluetongue and both died.

These results occasioned some surprise, since both the Mimosa Park and Cyprus strains when tested in South Africa had produced an absolute immunity. It is possible that the material may have undergone some change during transit or storage, especially in the case of the Mimosa Park vaccine where, to judge by the results obtained, the virus may not have been viable at all. The egg-attenuated Cyprus strain, while not producing an absolute immunity in the tests described above, did at least give very considerable protection and it was expected that in the case of an outbreak of Bluetongue in the field this vaccine would prove to be of very great service in controlling losses.

When the disease broke out in October, 1946, vaccination of all unaffected and in-contact sheep in affected villages was commenced, using the egg-attenuated Cyprus strain, and when this was completed, all sheep in a zone ahead of the line of spread were similarly vaccinated. Just under 20,000 sheep were immunised. The disease appeared to have been completely suppressed by the middle of December, with a total death-roll of under 200 as compared with about 2,500 in the 1943 outbreak when no vaccine was available. Doubtless deaths would have been considerably fewer had the disease been notified more promptly. Although it was expected that some time would elapse before immunity developed, it was reported that

in actual practice no fresh cases occurred later than two or three days after the date of vaccination.

As in both 1943 and 1946 the disease had commenced in the same district, it was decided in 1947 that sheep in this area and other villages which had been heavily affected the previous year should all be vaccinated during September and October, without waiting for the disease to put in an appearance. Nearly 14,000 sheep were injected. In spite of this precaution, 16 cases with two deaths occurred in animals said to have been vaccinated. Most of the cases were very mild. In four other areas where the sheep had not been vaccinated and where the disease had not occurred in the previous outbreaks apart from a few sporadic cases, 46 cases were reported with eleven deaths.

A sample of virus from a case in the 1946 outbreak was forwarded to Onderstepoort, and was found to be antigenically distinct from that isolated from the 1943-44 outbreak (Alexander, 1948). When twelve sheep immune to the first strain were challenged with the second, six had no immunity, three gave doubtful reactions and only three were immune. The presence of more than one distinct strain naturally occurring in Cyprus is probably the cause of the occasional failures of the vaccine to give full protection in the field.

DISCUSSION

During 1943-44, studies were made on the virus of Bluetongue which had previously been seen in a number of outbreaks among sheep in Cyprus without having been recognised as such. Attempts to attenuate the virus by passage, as had been done by Theiler and Du Toit, were unsuccessful. In the earlier passages, very large amounts of virulent blood were given intravenously, for fear that if too small doses were injected the animals might fail to react and the strain die out. On the other hand the large size of the dose may have been responsible for a loss in virulence of the virus. The dose was therefore gradually decreased as the experiments continued, until finally the amount of oxalated blood used was equivalent to 3·5 c.c. of undiluted blood. In the last four generations of each strain the dose was given subcutaneously.

In Theiler's attenuation experiments he had no deaths after the tenth generation, and Du Toit had none after the eleventh. However, De Kock *et al.* (1937) found a strain of the virus which they were unable to attenuate by serial passage through sheep (the "Tzaneen" strain). Neitz (1948) has concluded that this was normal, and has expressed the opinion that the results of the experiments of Theiler and Du Toit did not represent a true attenuation but merely an adaptation to artificial passage, and that their strains were of low virulence to begin with. The apparent commencement of attenuation in these Cyprus strains of the virus was probably illusory, and the variation in severity of the reactions may have

been connected with climatic or other external conditions. Neitz and Riemerschmid (1944) have shown that exposure of inoculated sheep to solar radiation can cause severe reactions even to vaccine strains of the virus. It was noticed during the Cyprus experiments when two or more sheep were being inoculated each week with two different strains, that there was on many occasions a close resemblance between the reactions of sheep inoculated at the same time in spite of different material being used. One week both strains would cause unusually high temperatures ; another week both strains would evoke non-febrile reactions. One week the highest temperature reached was on the seventh day in both animals ; the next it was the day following inoculation. One week the salivation in both animals was more profuse and frothier than any seen previously except in a few natural cases ; another week, when three animals were inoculated, the saliva was thick and sticky in all three. In 17 successive weeks, only six times did one sheep die and the other recover, and even then certain similarities were noted in three pairs, although the outcome was different. In the remaining cases either both died (in one case three died) or else both recovered (in one case four recovered). During the 18th week, after three generations of mild reactions with recoveries, four animals were inoculated and three reacted severely and died. Unfortunately no records were kept of climatic conditions at the time, but it is unlikely that these resemblances between the reactions to the two strains on so many occasions were mere coincidence. The apparent variations in virulence may have been due to the same type of cause.

Different outbreaks of the disease varied greatly in virulence. This does not appear to be correlated with the condition of the animals, for immediately prior to the 1943 outbreak which was the most virulent ever seen, all the flocks in the Island were in exceptionally good condition. This variation may have been due to different strains of virus having been involved ; or it may have been correlated with some combination of climatic factors, as suggested above in connection with the variation between the reactions of individual animals inoculated at different times. Unfortunately this variation in severity between one outbreak and another makes it difficult to assess with certainty the benefits conferred by the vaccine. For example, an outbreak such as that of 1946 which was apparently cut short by vaccination might possibly have died out spontaneously even in the absence of control measures.

Recent workers on Bluetongue (Alexander *et al.*, 1947) have called attention to the fact that strains of the virus can sometimes be inoculated into sheep without causing any apparent reaction ; but that if blood from the non-reactor is subinoculated into another a week later, a reaction will be obtained which, if not typical, will probably give a reaction when passed on to yet a third animal. Had this been done with some of the apparent failures in the trans-

mission tests described here, *e.g.*, the sheep in 1939 which was inoculated with spleen emulsion from a fresh Bluetongue carcase and several of the 1943 animals inoculated with filtrates, reactions might have been obtained. Similarly, if passage had been continued from the animals inoculated with filtrates of putrid blood, the virus might perhaps have been shown to have maintained its virulence to the full.

SUMMARY

A number of outbreaks of Bluetongue have been observed in sheep in Cyprus at recurrent intervals since 1924. The most serious was in 1943 when the virus was isolated and identified.

The symptoms observed in Cyprus are described and correspond closely with those described from South Africa.

The virus was studied in the laboratory, but attempts to attenuate it by serial passage through sheep were unsuccessful.

Various vaccine strains received from Onderstepoort were tested, but the only one which gave satisfactory protection under Cyprus conditions was the original Cyprus virus attenuated at Onderstepoort by serial passage through chick embryos. This egg-attenuated vaccine was used with success in subsequent outbreaks of the disease in 1946 and 1947.

ACKNOWLEDGMENTS

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OBSERVATIONS ON THE MODE OF ACTION OF SULPHANILAMIDE AND PENICILLIN ON *STR. AGALACTIAE* IN MILK

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INTRODUCTION

Since injecting one of the sulphonamides or penicillin in various concentrations into the udder *via* the teat is the form of therapy now employed in bovine streptococcal mastitis, it was thought that a study of the effect of these drugs by contact *in vitro* with *Str. agalactiae* might throw light, not only on their mode of action, but also on their effective therapeutic concentration within the udder.

For the purpose of these *in vitro* studies, milk was chosen since it is a suitable test medium for promoting rapid growth of *Str. agalactiae*, a factor which is essential for the study of sulphonamide and antibiotic action; moreover, studies on such a medium could be related directly to the therapeutic action of these substances in the udder.

Experiments with Sulphanilamide

Materials and Methods

The general procedure adopted in these experiments was to inoculate milk containing varying concentrations of suphanilamide with a suitable inoculum of a freshly isolated strain of *Str. agalactiae*. Thereafter the milk was incubated at 37° C. and at intervals one drop (·02 ml.), was delivered from a dropping pipette into duplicate roll tubes containing blood-agar. These tubes were incubated for 48 hours and the number of colonies subsequently developing from the inoculum was counted.

In order to give a suitable number of colonies for counting in roll tubes, a 1 : 100 dilution of the freshly grown (18 hour) Hartley broth culture of the streptococcus was made in sterile saline and then 0·1 ml. of the dilution was added to 9·9 ml. milk. By this technique it was found that a roll tube count of approximately 200 colonies was obtained from one drop of milk and a total initial count of approximately 10,000 organisms per ml. The concentrations of sulphanilamide selected which were 1 : 500 (200 mg. per cent), 1 : 5000 and 1 : 50,000 were found to contain the range present in milk secreted from udders after previous injection of a therapeutic dose, namely 50 ml. of a 30 per cent. suspension.

RESULTS

A preliminary experiment was carried out to ascertain the effect of the drug when in contact with *Str. agalactiae* for a period ranging from 1 to 8 hours in milk which had been sterilised by successive steamings. Since no bacteriostatic action was observed, further experiments were designed to include milk freshly removed from the udder in which the leucocytic action of the cells present might be expected to enhance the bacteriostatic action of the drug. The results of this experiment are shown in Table I.

TABLE I

THE ACTION OF SULPHANILAMIDE IN VARIOUS CONCENTRATIONS ON *Str. agalactiae* IN FRESH AND STERILISED MILK AT 37° C.

Medium	*Time of contact*	*Sulphanilamide concentration*			*Control*
		1 : 500	1 : 5,000	1 : 50,000	
Fresh	1 hour	93	83	100	94
Sterilised		100	110	141	164
Fresh	2 hours	117	150	80	82
Sterilised		270	410	337	393
Fresh	3 hours	108	101	91	102
Sterilised		631	1004	631	1,060
Fresh	4½ hours	71	110	88	83
Sterilised		In.	1,746	1,890	In.
Fresh	6 hours	144	109	194	107
Sterilised		In.	In.	In.	In.
Fresh	8 hours	230	145	118	200
Sterilised		In.	In.	In.	In.

The figures in the columns show the number of colonies present in 1 drop (0·02 ml.). In. = Innumerable colonies.

It will be seen that, not only were different sulphanilamide concentrations without effect on the number of organisms but that the development of the organisms was different in fresh and in sterilised milk. In the former, they did not multiply even in the control medium, while in the latter growth developed rapidly. To explain the difference in growth rate it must be concluded that there is a natural bacteriostatic factor in fresh milk which is effective in checking growth of *Str. agalactiae* for a period of at least 8 hours.

The inhibitory effect of milk on bacteria which was first recognised by Hesse (1894) and by Rosenau and McCoy (1908), was further investigated by Jones and Simms (1929), who showed that the inhibitory principle in fresh milk was heat labile and delayed the growth of mastitis streptococci for 5 to 6 hours. In the present experiment, the bacteriostatic action of sulphanilamide was masked

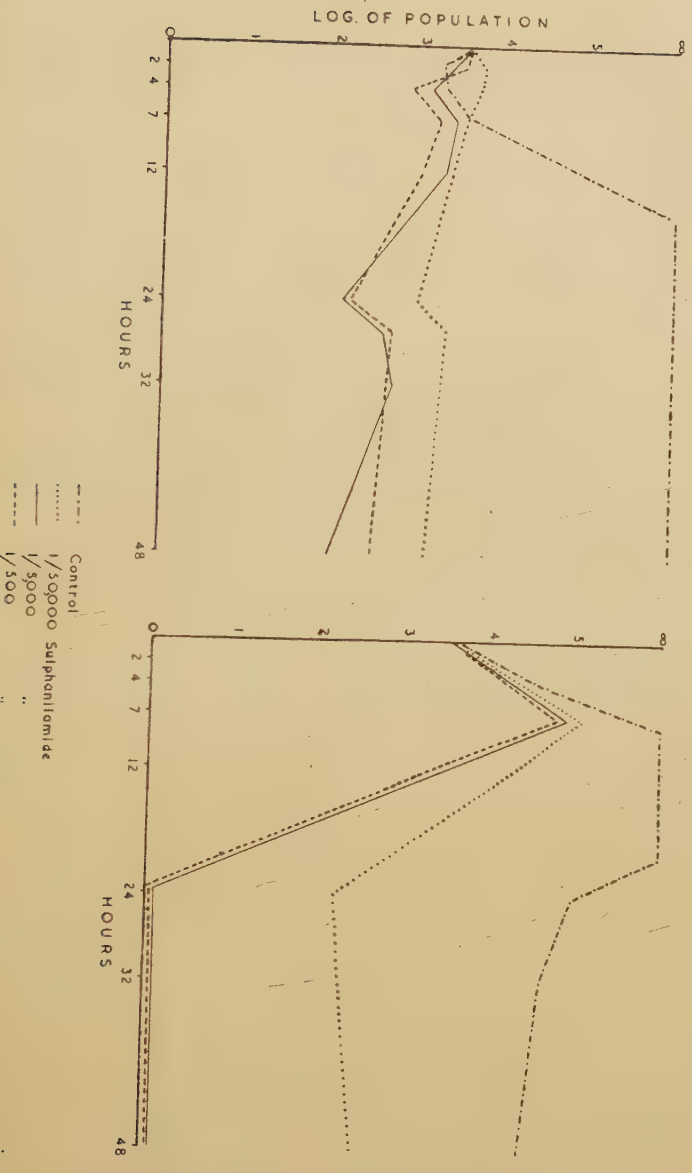


Fig. 1

EFFECT OF SULPHANILAMIDE ON STR. AGALACTIAE

by this factor during the selected short period of contact. It was decided therefore to extend the time of contact in both milks to 48 hours.

Prolonged Contact

A suitable dilution of a culture of a freshly isolated strain was inoculated as before into milk freshly drawn from a non-infected cow and also into the same milk heated at 90° C. for 10 minutes,

a temperature sufficient to destroy the natural inhibitory factor. Roll tube counts of the incubated milks were made and the results, obtained at successive intervals up to 48 hours, are represented graphically in Fig. 1.

In the control fresh milk it is seen that *Str. agalactiae,* after a lag period up to the 7th hour of incubation, enters the logarithmic phase with increased counts at the 12th and 24th hours. All concentrations of sulphanilamide inhibited growth but were not bactericidal even at the 48th hour. On the other hand, no lag period was apparent in the heated milk and the rate of multiplication of the organism in all concentrations of sulphanilamide was the same as in the control milk up to the 7th hour, after which there was a progressive fall. By the 24th hour, no organisms could be recovered from the milks containing 1 : 500 and 1 : 5,000 sulphanilamide while a low count (200 per ml.) was found in the milk containing 1 : 50,000 concentration. The results described are typical of those obtained in the case of six freshly isolated strains of *Str. agalactiae.* Three of these strains belonged to the serological type "a" and were sensitive to sulphanilamide (1 : 2,000) in blood-agar, while three were "b" type resistant strains.

These experiments, therefore, demonstrate the lag period during which sulphanilamide appears to have no action on *Str. agalactiae.* While the action of normal fresh milk in preventing the development of the organisms for at least 7 hours may mask the inaction of sulphanilamide for a similar period, proof of the delay in sulphanilamide action can be obtained in milk which has been heated to destroy this inhibitory factor.

It has been noted by several workers (McIntosh and Whitby, 1939; Hadley and Hadley, 1941; Neter, 1941) that sulphanilamide action is delayed during the early period of contact, but no definite evidence has been produced to explain this delay. Since the bactericidal effect of sulphanilamide was exhibited only while the organisms were in an active state of multiplication, it seemed likely that organisms in this phase were more susceptible to the action of the drug than those which were not dividing. The explanation of the mode of action of the sulphonamides on bacteria is founded on the work of Woods (1940) and of Fildes (1940) who propounded the theory that sulphanilamide acts by interfering with the metabolism of the organism. The metabolic process concerned is the utilisation by the bacterial cell of para-amino-benzoic acid (P.A.B.A.), a product essential for the growth of some organisms. In the presence of excess sulphanilamide, the enzyme concerned with the utilisation of this essential metabolite may be subjected to the competitive action of the drug, which is structurally closely related to P.A.B.A., with the result that the growth is inhibited. As has been demonstrated by Brownlee (1945) the degree of this competitive action and, therefore, the efficiency of the drug, is further related to the P.A.B.A. content of the test medium. According to this author milk contains virtually none of this amino acid and therefore the organism must depend on its own power of synthesis for satisfying its growth requirements.

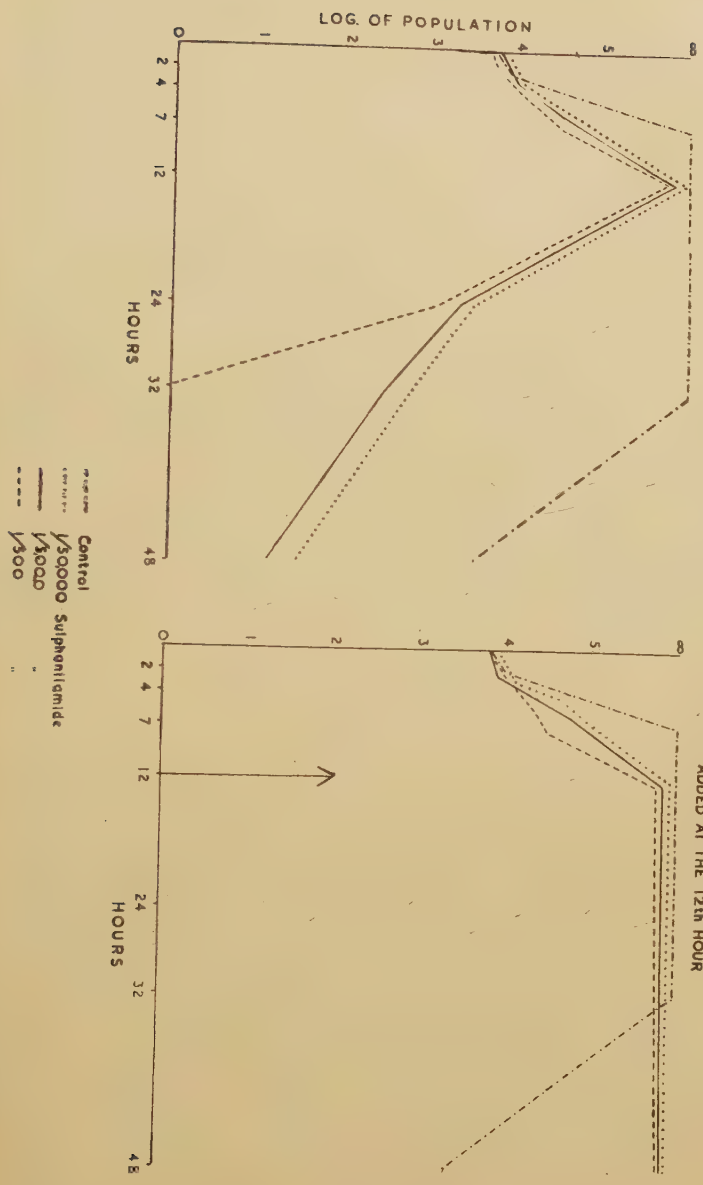


Fig. 2

EFFECT OF SULPHANILAMIDE ON STR. AGALACTIAE

The Effect of Adding Para-amino-benzoic acid

In order to test the value of the above theory in relation to the results obtained with *Str. agalactiae* sufficient P.A.B.A. was added to satisfy its growth requirements at the critical period when the bactericidal action of sulphanilamide began in heated milk. Various workers have attempted to define quantitively the ratio between the sulphanilamide content and that of P.A.B.A. which, if present in

a growth medium, will reverse the bacteriostasis of the former (McLeod, Mayr-Harting and Walker, 1944; Lowell, Strauss and Finland, 1941; Wood, 1942). In general, it has been found that one part of P.A.B.A. will neutralise about 2,000 parts of sulphanilamide.

In the following experiment, P.A.B.A. was added to milk containing 1 : 500, 1 : 5,000 and 1 : 50,000 concentrations of sulphanilamide in the ratio of one part P.A.B.A. to 1,000 parts of sulphanilamide. The addition was made after 12 hours incubation at 37° C. The results of this experiment are shown in Fig. 2. In the control milk and in milks containing different sulphanilamide concentrations, *Str. agalactiae* multiplied until the 12th hour. Thereafter, while the milk count in the control remained high, that in the sulphanilamide series decreased and by the 48th hour, few organisms remained. On adding P.A.B.A. at the 12th hour, however, no such decrease in count took place and the organism continued to multiply actively.

It can be concluded, therefore, that the delay in sulphanilamide action may be explained by the fact that the drug only becomes effective when the multiplying organism is unable to synthesise P.A.B.A. at a sufficient rate to antagonise the competitive action of sulphanilamide.

Experiments with Penicillin

Materials and Methods

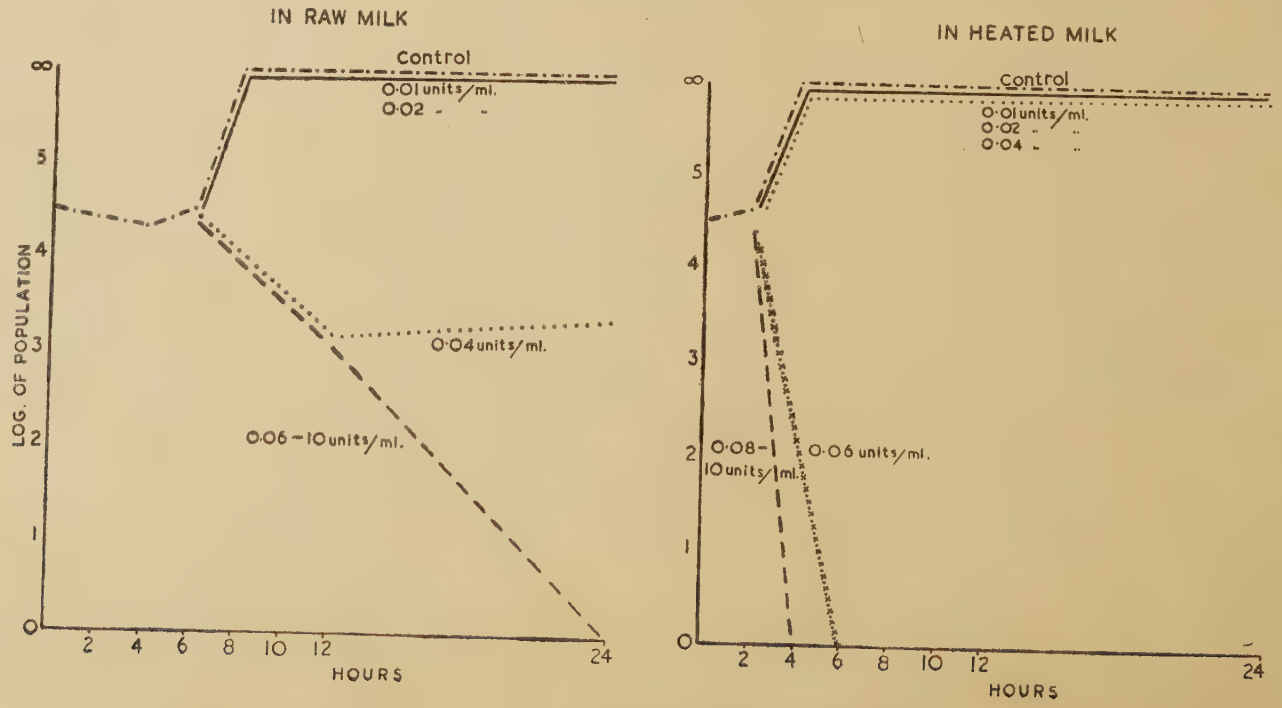
The same general procedure as described in the case of sulphanilamide was employed for determining the action of penicillin on *Str. agalactiae* in milk. The calcium salt of penicillin, obtained in the form of tablets each containing 10,000 Oxford units, was used, the potency of the batch employed being 438 units per mg. A solution containing 100 units per ml. in sterile distilled water was prepared from which further dilutions of 1, 2 and 50 units per ml. were made. Concentrations varying from 0·01 to 10 units per ml. were chosen since previous workers had shown that the sensitivity of most strains of *Str. agalactiae* fell within this range. (Brownlee, 1945; Watts and McLeod, 1946; Heishman, 1947). The growth of five freshly isolated strains was studied in the presence of the above concentrations of penicillin at 37° C. in fresh milk and in milk heated to 90° C. for 10 minutes. The effect of contact at 18° C. was noted in the case of two strains in heated milk only.

RESULTS

At 37° *C.*—All five strains gave similar results and the general pattern is represented graphically in Fig. 3. It will be seen that the action of penicillin differs in the fresh and heated milk. While no increase in count was noted in the control fresh milk during the first 6 hours of incubation, due to the natural lag previously observed, the organism entered the logarithmic phase of growth after this

FIG. 3

THE EFFECT OF PENICILLIN IN VARYING CONCENTRATIONS ON THE GROWTH OF STR. AGALACTIAE AT 37°C.



interval. At this time also, differences were first observed in the growth in milk containing penicillin. Concentrations of 0·01 and 0·02 had little effect, whereas 0·04 units reduced the growth and 0·06 to 10 units were bactericidal in 24 hours. In fresh milk, the efficacy of 10 units per ml. in this respect was no greater than that of concentrations as low as 0·06 units per ml. In the heated milk, no distinct lag period in the growth of the organism in the control milk was observed and a sharp demarcation between completely bactericidal concentrations and those which were ineffective was demonstrated after contact for only 6 hours.

At 18° *C.*—It was found that the action of penicillin at this temperature was less effective than that observed at 37° C. Two strains which were destroyed by 0·06 units per ml. in 6 hours at 37° C. survived exposure to 10 units per ml. for 48 hours at 18° C. The results obtained in the case of a typical strain are shown in Table II.

The action of penicillin was therefore directly related to the phase of activity of the test organism, an observation first noted by Hobby, Meyer and Chaffee (1942) and confirmed by Rantz and Kirby (1944).

TABLE II

THE ACTION OF PENICILLIN ON *Str. agalactiae* AT 37° C. AND 18° C.

Sample	*Time of contact (hours)*	*Control*	*Penicillin—units per ml.*									
			0·01	0·02	0·04	0·06	0·08	0·1	0·2	1	5	10
	0	380										
37° C.	6	In.	In.	In.	353	0	0	0	0	0	0	0
18° C.		366	367	341	362	335	326	286	351	165	229	242
37° C.	12	In.	In.	In.	In.	0	0	0	0	0	0	0
18° C.		563	406	451	399	383	332	227	278	200	166	127
37° C.	24	In.	In.	In.	In.	0	0	0	0	0	0	0
18° C.		In.	42	43	43	86	36	21	25	15	1	12
18° C.	36	In.	20	4	16	11	14	16	22	0	9	7
18° C.	48	In.	6	3	3	0	7	3	3	3	1	4

Counts per 0·02 ml. after contact with varying concentrations in heated milk.
In. = Innumerable colonies

Sensitivity of Strains to Penicillin

The sensitivity of 25 freshly isolated strains of *Str. agalactiae* was examined by a standard technique. The strain was grown for 18 hours in Hartley's broth at 37° C., the culture diluted 1 : 100 in sterile saline and then 0·1 ml. added to 9·9 ml. sterilised separated milk

containing penicillin concentrations of 0·01 to 0·2 units per ml. The number of organisms present in 1 ml. of test medium was approximately 20,000 when assessed by the original counts of five strains. Roll tube counts were made after 24 hours' incubation at 37° C. by removing 0·02 ml. into a standard volume of blood agar. At this time it was possible to differentiate clearly between bactericidal concentrations and those which had not impeded the development of the organism under test.

The results of these tests are given in Table III which shows that

TABLE III

SENSITIVITY OF *Str. agalactiae* TO PENICILLIN IN HEATED MILK

Strains tested	*Number of strains sensitive to concentrations*			
	0·01–0·02 *units per ml.*	0·04–0·06 *units per ml.*	0·08–0·1 *units per ml.*	0·2 *units per ml.*
25	0	20	4	1

the majority of *Str. agalactiae* strains are sensitive to concentrations of 0·04 to 0·06 units per ml. when tested by a technique designed to test the effect on a relatively small number of organisms. These findings confirm those of Brownlee (1945) and Heishman (1947) who found that most strains were of uniform sensitivity and that the lethal concentrations ranged from 0·06 to 0·12 units per ml. Further, the uniformity of this end point is such that it can be adapted to the estimation of penicillin concentration in milk.

Estimation of Penicillin by a Serial Dilution Technique

In the routine examination of milk samples for the presence of residual penicillin following udder injection, the Oxford plate method devised by Florey *et al.* (1941) and further developed by Heatley (1944) has been found very convenient. In this laboratory, the test has been found to be accurate when standard concentrations of penicillin, *viz.*, 20, 10, 2 and 1 unit per ml., have been examined separately on several occasions by a standard technique: further, since a large agar surface of regular depth can be set up within wooden frames, the test allows a large number of samples to be tested simultaneously. Although Foster and Woodruff (1943) and Hayes (1945) commend this method of assay for the same reasons, they point out certain irregularities which may be encountered due to (1) a period elapsing before prepared plates are incubated; (2) unevenness in the depth of the agar in the plates. While it may be held that these are defects of this method of assay, their occurrence can be corrected by adopting standard conditions.

It has been found in practice that a more serious disadvantage of the plate method is its failure to detect low concentrations of

TABLE IV

SERIAL DILUTION TEST USING STRAIN 139

Dilution	1 : 1	1 : 2	1 : 4	1 : 8	1 : 16	*Control*
Penicillin conc. (units per ml.)	0·2	0·1	0·05	0·025	0·0125	0
Presence of growth at 48 hours	—	—	—	+	+	+

penicillin with accuracy. For this reason a serial dilution method based on the foregoing results has been devised, the general procedure being similar to that adopted by Fleming (1929), Selbie, Simon and McIntosh (1945), and Watts and McLeod (1946). An initial dilution of 0·4 units per ml. of penicillin was prepared from a sterile aqueous solution of 2 units per ml. by adding 0·5 ml. to 2 ml. of sterile separated milk. From this, twofold dilutions were made in 1 ml. volumes of milk contained in sterile plugged 3 in. × ½ in. tubes. To each tube was added one drop (0·02 ml.) of a 1 : 100 dilution of an 18-hour Hartley broth culture of a strain found sensitive, by previous standard roll tube tests, to 0·04 units per ml. of penicillin. Into each milk tube was also placed one drop of 1 : 1,000 sterile solution of brom-cresol-purple to indicate change in colour due to the acid development in the presence of growth. The tubes were incubated at 37° C. for 48 hours, at which time the results could be clearly read.

The result shown in Table IV was regularly obtained by the technique described above with the test strain. In order to compare the accuracy of this test in the determination of penicillin

TABLE V

ASSAY OF PENICILLIN CONCENTRATIONS IN PREPARED MILK SAMPLES BY (1) SERIAL DILUTION; (2) THE OXFORD PLATE

Actual concentration	*End points by serial dilution in milk*								*Values obtained by:*	
	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	*Tube test*	*Plate test*
1 unit/ml.	—	—	—	—	—	+	+	+	1·6	1·0
0·8 unit/ml.	—	—	—	—	+	+	+	+	0·8	0·7–1
0·4 unit/ml.	—	—	—	+	+	+	+	+	0·4	0·47
0·2 unit/ml.	—	—	+	+	+	+	+	+	0·2	Inhibitory zone indefinite
0·1 unit/ml.	—	+	+	+	+	+	+	+	0·1	
0·05 unit/ml.	+	+	+	+	+	+	+	+	0·05	No zone

content with the Oxford plate method, an experiment was carried out using samples containing known low concentrations. It can be seen from Table V that the results obtained by titration in milk gave almost exact values for the concentrations under test, whereas accuracy could not be obtained by the plate method with concentrations below 1 unit per ml. The ring diameters obtained with concentrations below 0·4 units per ml. cannot be measured accurately and hence cannot be interpolated from the straight line which ordinarily gives accurate values from 1 to 100 units on logarithm paper No. 9 L. The uniformity of the results by this serial dilution method in milk suggested that it might be applied to the assay of penicillin in blood.

Effect of Serum on Penicillin Estimation

Many tests have been employed for the determination of the penicillin content in blood serum and these have been fully described by Fleming (1946).

These tests are dependent on the inhibition of either a sensitive streptococcus or a staphylococcus, by dilutions of serum which are kept in contact by either the slide cell method or the capillary tube method. In the interpretation of the results, workers have generally not attributed any interference with the bactericidal property of penicillin to the presence of serum, since it has been understood in general that it does not possess any prejudicial effect. The results of experiments carried out by Bigger (1944), however, showed that both serum and blood inactivated penicillin and that the degree of inactivation varied greatly with different samples of serum, but was always much greater at 37°C. than at lower temperatures. The concentration of serum when inactivation was most marked was as high as 50 to 90 per cent., while lower concentrations produced less effect. In the recent work of Watts and McLeod (1946), however, it was found that concentrations of 1 to 10 per cent. of fresh bovine serum reduced the activity of penicillin against *Str. agalactiae* during contact for 72 hours in litmus milk, but that higher concentrations produced less inactivation. Further, it was found that the anti-penicillin factor present in serum was destroyed by heating at 56° C. for 30 minutes.

In view of the foregoing conflicting evidence two experiments were designed to test the effect of the presence of serum on the serial dilution test as applied to the estimation of small concentrations of penicillin in blood. In the first experiment three concentrations of penicillin namely, 5, 1 and 0·1 units per ml. were prepared in fresh bovine serum and also in serum heated at 56° C. for 30 minutes, and progressive twofold dilutions of each were made in sterilised milk. A similar series of concentrations were made in milk to serve as a control. The same amount of culture dilution of strain 139 (·02 ml. of 1 : 100 dilution of Hartley broth culture) was inoculated into each dilution and also one drop of 1 : 1,000 sterilised brom-cresol-purple solution. The results of the first experiment, read after 48 hours' incubation, are shown in Table VI.

TABLE VI

ESTIMATION OF PENICILLIN IN SERUM BY SERIAL DILUTION IN MILK

Sample	*Penicillin units per ml.*	*End points obtained by dilution in milk*							
		1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256
Fresh serum	5	—	—	—	—	—	—	—	—
	1	—	—	—	—	—	—	+	+
	0·1	—	—	+	+	+	+	+	+
Heated serum	5	—	—	—	—	—	—	—	—
	1	—	—	—	—	—	—	+	+
	0·1	—	—	+	+	+	+	+	+
Milk control	5	—	—	—	—	—	—	—	—
	1	—	—	—	—	—	—	+	+
	0·1	—	—	+	+	+	+	+	+

It will be seen that the end points of similar concentrations of penicillin in either fresh bovine serum, heated serum or milk were identical. This finding affords no evidence of any inhibitory action of serum, but it may be pointed out that in this preliminary experiment there was a diminishing concentration of serum as dilution progressed, so that higher dilutions included such small concentrations of serum that no inhibitory effect might be apparent.

To offset the possible "diluting out" of an inhibitory factor, a further experiment was designed in which the concentration of serum ranged from 0·4 to 50 per cent. while the penicillin varied from 1 to 0·0125 Oxford units per ml. The results (Table VII) showed that fresh serum did not affect the bacteriostatic activity of penicillin, a finding which does not agree with that of Watts and McLeod (1946) who used the same technique. It seems, therefore,

TABLE VII

THE EFFECT OF VARYING CONCENTRATIONS OF BOVINE SERUM ON THE BACTERIOSTATIC ACTIVITY OF PENICILLIN IN MILK

Concentration of serum (%)	*Control no penicillin*	*Penicillin in Oxford units per ml.*							
		1	0·8	0·4	0·2	0·1	0·05	0·025	0·0125
50	+	—	—	—	—	—	—	+	+
25	+	—	—	—	—	—	—	+	+
12·5	+	—	—	—	—	—	—	+	+
6·25	+	—	—	—	—	—	—	+	+
3·1	+	—	—	—	—	—	—	+	+
1·6	+	—	—	—	—	—	—	+	+
0·8	+	—	—	—	—	—	—	+	+
0·4	+	—	—	—	—	—	—	+	+
0·0	+	—	—	—	—	—	—	+	+

that this serial dilution test in milk using a sensitive *Str. agalactiae* strain gives reliable results not only for assaying small concentrations of penicillin in milk but also in serum.

SUMMARY

The results reported in this paper may be considered to contribute further information on the mode of action of two therapeutic substances now widely employed for combating pyogenic infection. By studying the "*in vitro*" action of sulphanilamide and penicillin on *Str. agalactiae* in its natural medium, namely milk, it has been found that certain essential features in their mode of action are related to the stage of activity of the organism.

The action of sulphanilamide was found to be different in fresh milk from that in milk heated to 90° C. for 10 minutes. In the former, the drug was bacteriostatic in concentrations of 2 to 200 mg. per cent whereas in heated milk, following a lag period of 7 to 12 hours during which the organism actively multiplied, the drug was progressively bactericidal. Using the Woods-Fildes theory as a basis to explain the delay in sulphanilamide action, it was found that by supplying para-amino-benzoic acid to the medium at the 12th hour in the ratio of one part P.A.B.A. to 1,000 parts sulphanilamide, growth was not impeded. The action of penicillin was also found to be directly related to the phase of activity of the organism. In fresh milk, where growth was delayed for 6 hours, the organism was destroyed in 24 hours by concentrations of 0·06 to 10 units per ml. whereas in milk heated to 90° C. for 10 minutes this result was obtained within 6 hours.

While the action of the two drugs was similar in so far as they were more effective against the multiplying organism, it was noted that whereas *Str. agalactiae* multiplied actively in the presence of sulphanilamide so long as the organism was able to synthesise P.A.B.A. and the enzyme concerned with its utilisation was able to resist the competitive inhibition of the drug, no multiplication took place in the presence of lethal concentrations of penicillin. It would appear, therefore, that the action of penicillin is not related to its power to replace a metabolite essential to the growth of the organism but to its direct destructive action at the time when the organism begins to multiply.

Further, it was found that the rate of destruction of *Str. agalactiae* by penicillin did not increase with concentration in the milk since no more rapid bactericidal action was produced by concentrations greatly above the minimal effective concentration of 0·04 to 0·08 units per ml. This finding, which was particularly well demonstrated by contact in fresh milk, may have some influence on the selection of dosage and interval between the repeated injections now applied in the therapeutic treatment of bovine mastitis.

A serial dilution test in milk is described for assaying small concentrations of penicillin which was found to give more accurate results than the Oxford plate method. It was found that the presence of serum did not influence the accuracy of this test.

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THE EFFECT OF "WHITE SCOUR" ON THE ABSORPTION OF VITAMIN A BY CALVES

By

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INTRODUCTION

In papers dealing with "White Scour" disease of young calves reference has often been made to impaired absorption of vitamin A and carotene. Thus, Esh, Sutton, Hibbs and Krauss (1948) recorded a fall in the vitamin A concentration in the plasma in newborn calves, which coincided with the onset of scouring. Similar observations on the level of carotene in the plasma were made by Spielman, Thomas, Loosli, Norton and Turk (1946). The effect of scouring on liver storage of vitamin A has also been investigated; Esh *et al.* (1948) found smaller liver reserves in scouring animals than in normal animals.

Lowered levels of vitamin A or carotene in the blood do not necessarily indicate an impaired absorption, but may be caused by other factors such as increased utilisation of the vitamins due to pyrexia or other causes. Reduced liver storage and increased excretion of vitamin A in the faeces, do however, provide confirmatory evidence of an impaired absorption. In the experiments reported here, all three criteria were used on the same animals in order to study the absorption of vitamin A in "White Scour." The levels of vitamin A in blood before and after administration of massive doses were examined in healthy and scouring calves, while in some of the animals the loss of unabsorbed vitamin A through the faeces and the percentage of dose stored in the liver were also estimated. An account of some of our earlier experiments has been included in a preliminary report by Blakemore, Davies, Eylenburg, Moore, Sellers and Worden (1948).

EXPERIMENTAL PROCEDURE

Treatment of Animals

Breed and diet. Seventeen newborn calves of Ayrshire, Jersey and Friesian breeds were obtained from farms in Cambridgeshire. The calves were removed from the dams immediately after birth, to prevent them obtaining colostrum and they were fed twice a day on reconstituted separated milk, mixed with 3 per cent. of arachis oil in a Waring Blendor. The amount given was two to four litres per day according to the weight of the animal. The first ten calves were given supplements of all the necessary B vitamins in the quantities given by Spielman, Loosli, Thomas, Norton and Turk

(1946). Since their administration had no noticeable effect either on the absorption of vitamin A or on the survival times of the calves, these supplements were not given to the remaining animals.

Incidence of scouring. Newborn calves remained healthy for the first two days of life and usually commenced scouring about the third day. The figures for control animals were obtained from calves which had not commenced scouring or in which scouring had been prevented by the injection of precolostrum (Blakemore *et al.*, 1948). Some animals were repeatedly dosed with vitamin A both before and after the incidence of scours and thus animals Nos. 1 to 5 appear both among the control and the scouring group according to their state of health.

Method of dosing. Halibut Liver Oil was used as a source of vitamin A for most animals; Nos. 13, 16 and 17 however, received a vitamin A concentrate prepared by Distillation Products Inc. The vitamin was emulsified in the Blendor with reconstituted milk (Eden and Sellers, 1949) in doses of 50,000 to 500,000 IU. In single dose experiments the total volume of the dose was about 200 ml., but in experiments where several doses were given, the dose was mixed with the morning feed.

Collection of materials for estimations. Blood was collected from the jugular vein before dosing and at various times after dosing. To prevent clotting the blood was mixed on collection with a 10 per cent. solution of potassium oxalate to give a final dilution of 0·1 per cent. of oxalate. The liquid nature of the faeces of scouring animals rendered the collection of total faeces impracticable, but estimations were made on representative samples. Representative liver samples were taken at slaughter or at death.

Chemical Methods

Methods of extraction. Plasma, 2 ml. samples, were treated according to the method of Yudkin (1941), based on the precipitation of proteins with ethanol and the extraction of the vitamin with light petroleum (B.P. 40 to 60° C.). Faeces, 2 to 5 g. samples, were dehydrated with anhydrous sodium sulphate and extracted with hot ether as in the method of Glover, Goodwin and Morton (1947). Liver, 2 to 5 g. samples, were treated according to the alkali digestion method of Davies (1933).

Estimation of vitamin A. The intensity of the blue colour in the antimony trichloride reaction for the estimation of vitamin A was measured in a single photocell absorptiometer (Eden and Sellers, 1949). Carotenoids were not estimated, as not more than traces were present in the materials examined.

RESULTS

Typical vitamin A absorption curve in control and scouring calf. Fig. 1 shows absorption curves of vitamin A in calves No. 11 and 12.

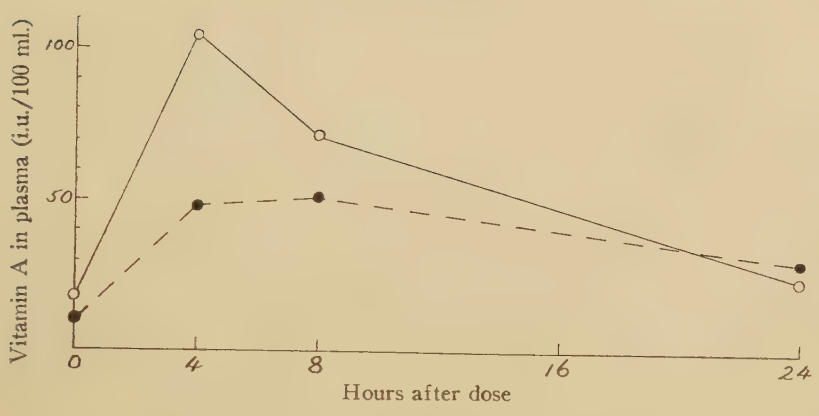

FIGURE 1
Changes of concentration of vitamin A in plasma after dosing.
o——o control animal (No. 11).
●——● scouring animal (No.12).

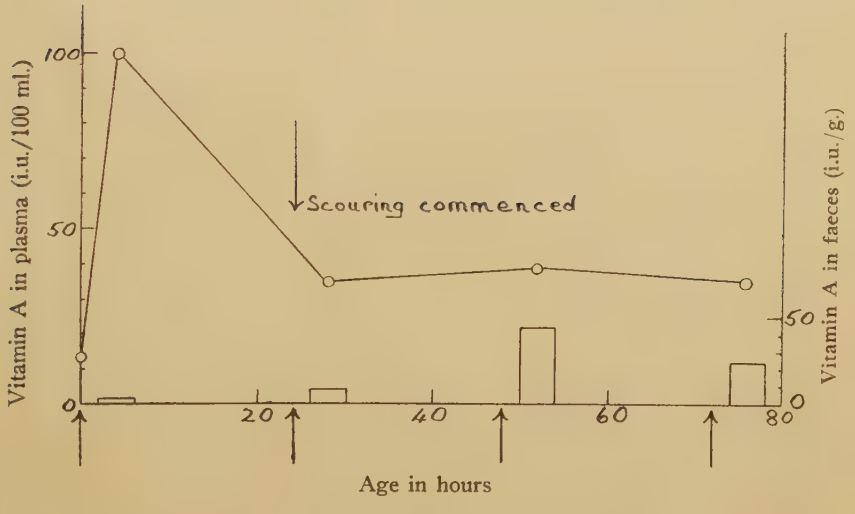

FIGURE 2
The effect of the onset of scouring on vitamin A absorption.
Samples of blood and faeces collected four hours after dosing (Animal No. 4).
o——o vitamin A in plasma.
□ vitamin A in faeces.
↑ administration of vitamin A.

The absorption peak occurred 4 hours after administration of the dose and the concentration of vitamin A in the blood at this point was in the control double that of the scouring animal. The shape of the curve resembled that obtained in humans suffering from impaired absorption (Adelsberg and Sobotka, 1943; Darby, Kaser and Jones, 1947).

Postabsorptive vitamin A levels in control and scouring calves. Since too frequent withdrawal of blood was impracticable in newborn calves complete curves were not obtained for all animals, but blood samples were collected before and 4 hours after dosing when the absorption was at its maximum. The data are presented in Table I. There were rises of 300 to 500 per cent. in the level of

TABLE I

CONCENTRATION OF VITAMIN A OF PLASMA AND FAECES IN CONTROL AND SCOURING CALVES AFTER DOSING

Calf number	Age in days	Vitamin A: Dose: Total I.Us. administered	Vitamin A: Plasma I.U./100 ml.: Before dosing	Vitamin A: Plasma I.U./100 ml.: Four hours after dosing	Vitamin A: Plasma I.U./100 ml.: Increase	Vitamin A: Faeces I.U./g.: 4 hours after dosing
			Control calves			
1a	14	500,000	22	110	88	3
2a	14	500,000	24	130	106	3
3a	1	100,000	23	94	71	3
4a	1	100,000	13	99	86	1
5a	1	100,000	19	86	67	—
10	3	75,000	33	102	69	—
11	3	59,000	19	104	65	2
13	2	175,000	34	202	168	2
16	2	150,000	—	243	—	1
17	2	150,000	15	96	81	0
			Scouring calves			
1b	36	500,000	21	18	3	37
2b	21	500,000	16	16	0	192
3b	2	100,000	—	30	—	98
3c	3	100,000	—	33	—	78
4b	2	100,000	—	34	—	9
4c	3	100,000	—	37	—	45
4d	4	100,000	—	34	—	25
5b	3	100,000	—	53	—	220
7	12	200,000	19	100	81	19
8	15	100,000	12	24	12	4
9	3	100,000	19	35	16	40
12	7	75,000	10	48	38	24
14	4	200,000	10	—	—	211
15	4	200,000	2	70	68	315

— = No estimations made.

vitamin A in the control animals whereas those in scouring animals were smaller or absent.

Vitamin A in faeces in control and scouring calves. Attempts were made to collect the total faeces in metabolism bags. As this proved impracticable after the onset of diarrhoea representative samples were collected 4 hours after dosing. Table I shows that the concentration of vitamin A in the faeces was higher in scouring than in control animals. Since the faeces of the scouring animals had a very loose and variable consistency, the difference in the vitamin A concentration between the two groups would be even greater when calculated on a dry weight basis.

Effect of onset of scouring on vitamin A levels. Some of the animals, Nos. 1 to 5, which were dosed repeatedly, remained healthy for a few days and then began to scour. The high blood levels observed after dosing while the animals were healthy were not reproduced once scouring had begun. The appearance of the vitamin A in the faeces coincided with the drop in the plasma levels (Fig. 2).

Liver storage. The reserves of vitamin A in the liver of newborn calves are very small (Spielman, *et al.*, 1946 b) and, as our experimental animals did not receive any other source of vitamin A besides that given in the dose, an estimate of the proportion of the dose stored in the liver could be ascertained at death. In experiments with single doses, about three times as much was stored in the liver of the controls as in the liver of the scouring animals

TABLE II

STORAGE OF VITAMIN A IN THE LIVERS OF CONTROL AND SCOURING ANIMALS

SINGLE DOSE EXPERIMENT

Calf number	*Killed hours after dose*	*Vitamin A*			
		Dose	*Liver*		
		Total I.Us. administered	*I.U. g.*	*Total I.U.*	*% Recovery of dose in liver*
		Control calves			
10	24	75,000	67	41,000	55
11	24	59,000	44	23,500	31
13	4	175,000	108	67,200	39
16	4	150,000	40	20,400	14
17	5	150,000	52	33,200	22
		Scouring calves			
7	4	200,000	16	13,000	15
12	24	75,000	18	13,500	18
14	24	200,000	20	9,500	6
15	12(D)	200,000	19	10,200	5

(Table II). In experiments with repeated doses, the figures showed (Table III) that provided the animals received some vitamin A before scouring had started, they were able to increase their liver reserves well above the levels at birth. Such increases were not found if vitamin A was withheld until scouring had commenced.

DISCUSSION

Various investigators have shown that several different factors may impair the intestinal absorption of vitamin A. Drugs, *e.g.* atropine which decreases intestinal tone, also decrease the absorption of the vitamin (Ingelfinger, Moss and Helm, 1943 ; Popper and Volk, 1944), whereas the reverse effect is observed when the intestinal tone is increased by cascara or neostigmine (Flax, Barnes and Reichert, 1942 ; Popper and Volk, 1944). Impaired absorption also results when the intestinal mucosa in man is damaged by tuberculosis lesions (Breese, Watkins and McCoord, 1942) or by the invasion of the protozoan Giardia (Katsampes, McCoord and Phillips, 1944). The gross thickening of the intestinal wall which occurs in bovines suffering from Johne's disease is also associated with poor absorption (Eden and Sellers, unpublished). In addition the vitamin is poorly absorbed when fat intake is defective as in sprue (Adelsberg and Sobotka, 1943 ; Darby, Kaser and Jones, 1947) or in coeliac disease (Kramer, Sobel and Gottfried, 1947 ; May and Lowe, 1948 ; Popper, Steigmann and Dyniewicz, 1948).

In the experiments described here defective absorption of vitamin A may have resulted from an increased intestinal movement and a consequent increased rate of passage of food in scouring calves.

TABLE III

STORAGE OF VITAMIN A IN THE LIVERS OF CONTROL AND SCOURING ANIMALS

REPEATED DOSE EXPERIMENT

Calf number	*Killed or died*	*Number of times dosed*	*Vitamin A*	
			Total I.Us. administered	*I.Us./g. of liver*
	Calves dosed before the onset of scouring			
1	K	2	1,000,000	170
2	K	2	1,000,000	60
3	D	3	300 000	82
4	D	4	400 000	278
5	D	2	200,000	120
9	K	2	200,000	38
	Calves scouring throughout whole period of dosing			
6	D	2	200,000	4
8	K	2	200,000	16

The actual involvement of these factors was deduced both from the clinical picture and from the demonstration of vitamin A in various regions of the intestinal tract. This increased rate of the passage of food would lower the amount of vitamin A absorbed, although other factors such as pathological changes of the mucosa may also have been involved. Further work is required to establish this point.

Impaired absorption of vitamin A can be improved in certain diseases by dosing vitamin A in special preparations (see Nutritional Reviews, 1948). In White Scour (Esh and Sutton, 1948) and in sprue (Adelsberg and Sobotka, 1943) improved absorption has been recorded when the vitamin was mixed with lethicin. In liver disease and coeliac disease of children absorption was improved when vitamin A was fed emulsified in water with "Tween" (a polyoxyalkylene derivative of sorbitan monalaurate) instead of the customary oily medium (Kramer *et al.*, 1947 ; May and Lowe, 1948 ; Popper *et al.*, 1948). Thus, it may be advisable to administer vitamin A in the form of a special preparation in order to enhance its absorption in scouring calves and to build up their reserves to avoid the possibility of the development of a subsequent deficiency. Experiments to investigate this possibility are being planned.

SUMMARY

Single or repeated doses of vitamin A were given by mouth to scouring and control calves. Vitamin A levels of plasma were determined before and after dosing. Vitamin A determinations were also made on the faeces and on the liver.

Four hours after dosing, the vitamin A level of the blood of all the control animals had risen by 300 to 500 per cent. The rise was much smaller in scouring animals and in some of them it did not occur at all.

In the control animals only traces of vitamin A were found in the faeces, whereas in the scouring animals definite evidence was obtained that vitamin A was lost in the faeces.

In the scouring animals the liver reserves of vitamin A were only about one third of those in the control animals.

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THE INCIDENCE OF ASPERGILLOSIS IN CAPTIVE WILD BIRDS

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INTRODUCTION

Aspergillosis in birds was first recognised during the latter half of the eighteenth century. In this country the first published record appears to be that by Richard Owen (1832) who, when dissecting a flamingo, observed "a green vegetable mould or *mucor*" lining "*vomicae*" in the lungs. Owen recognised the parasitic relationship and concluded that internal parasites are not derived exclusively from the animal kingdom. Since that time there have been many records of aspergillosis in man and animals, but particularly in birds, from all parts of the world (see Lesbouyries, 1941). In this country, where the disease is well known, there are a number of records, but the fungi involved have frequently not been examined critically and few observations have been made on the pathology of the disease. The present study is an attempt to make good this deficiency.

Avian aspergillosis is characteristically a disease of captivity. The data obtained have yielded information on the incidence of the disease in relation to the species of bird and the length of time in captivity, the identity of the fungi involved and the pathological changes induced. In addition, a few observations have been made on the relation of aspergillosis to tuberculosis.

MATERIALS AND METHODS USED

The cases recorded here are all those in which mycosis of any degree was found in birds at the Zoological Gardens in Regent's Park, London, during the two years from September, 1946, to September, 1948. All vertebrate animals dying in the Gardens are submitted to necropsy unless markedly decomposed. In the present series careful note was made of the type and site of the lesions and cultures and sections taken, except where decomposition rendered them unlikely to be of use. The cultures were all made on Sabouraud's or Czapek's medium. Paraffin sections were prepared and stained with haematoxylin and eosin and by Heidenheim's method. Gram's

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stain was used also at first, but no advantage found in it. Sometimes the features of the disease were seen better with one of the two stains, sometimes with the other.

The material from the Zoological Gardens was supplemented by cultures from poultry and other birds received at the Ministry of Agriculture's Veterinary Laboratory, Weybridge, from centres widely scattered throughout England and Wales.

MORBID ANATOMY

Distribution of Lesions. This is almost entirely in the respiratory system, and even here the incidence is highest in the air-sacs. The anterior thoracic ones are involved the most frequently and the most heavily. This localisation may depend on some mechanical factor in the arrangement of the air-passages as, for example, the fact that the ostia leading to them are larger than those to the other sacs, so that they receive more spores; alternatively, they may form a particularly favourable site for germination. However, in some cases other sacs than the anterior thoracics are involved more heavily.

Next in frequency come the lungs themselves, but these are seldom involved alone. In four cases (1, 2, 8 and 20), however, a single abscess only was found in a lung, and in one case (20) its true nature was only revealed by sections. The syrinx was involved twice (32 and 37), together with the lungs and air-sacs. In one case (28) mycotic granulomatous tissue filled the trachea when no lesions were found elsewhere. Two mycetomas of the mouth of uncertain aetiology are recorded (6 and 7).

In only three cases (3, 35 and 51) did the process appear to affect the abdominal viscera—and here direct spread was obvious—despite their proximity to some of the affected air-sacs. It can be stated emphatically that in birds, caseous lesions of the lungs are almost certain to be mycotic, while those of the liver and spleen are almost certain to be tuberculous. This is well seen in the cases of mixed infection recorded (12, 38, 70, 72, 75 and 76).

Appearance of the Lesions. This varies in accordance with their site. Those which are the easiest to recognise are found on the surfaces of the air-sacs. They consist of raised, white nodules on the serous surfaces which, even in their earliest stages, have a "woolly" surface different from that of tubercles. They may coalesce, producing large patches resembling white or greenish plush, while in the most advanced state of all, the whole sac becomes lined with the process. Then the surface appears to be rubbed down while the underlying tissues are fibrosed. The sac thus becomes converted into a stiff bag with walls which have lost all elasticity and have the appearance of felt, with a wrinkled, yellowish internal surface. It is obvious how markedly such a change must interfere with the function of the air-sac.

The second type of lesion is found in the lungs and resembles tuberculosis very closely. The lesions are scattered and generally

small with a white, necrotic centre, differing from that of the tubercle in being much more friable and in shelling out whole in all but the smallest lesions. The feel and appearance of the lesions is what would be expected from their composition of tangled hyphae, especially when opposed to the more homogeneous necrotic material found in the centre of a tubercle. The lesions of mycosis may coalesce to form large necrotic masses and sometimes the centres of these may become so liquid that they are hard to distinguish from a pyogenic abscess.

Sometimes, air-sacs which show no gross lesions, although such are present in other sites, contain a quantity of free, milky fluid containing large numbers of polymorphs, but no hyphae. This may possibly be an early stage of mycosis. We have found no free fluid in any air-sac with gross lesions in the walls.

MICROSCOPICAL APPEARANCES

A very wide range of appearances is found, but fundamentally, these can be referred to modifications of a quite simple pattern. The spores must gain entry by the respiratory passages, and wherever a lesion starts the mycelium spreads into the underlying tissues. The extent to which it does this varies much, however, depending on the consistency of the tissue. Thus hardly any deep spread occurs with lesions in the thin serous bags of the air-sacs. Here the surface endothelium is denuded and a layer of chronic granulation tissue is laid down. More superficial to this, a very thick layer of tangled hyphae is seen, sometimes arranged into definite arcades. The tangle gets looser as the surface is approached where a number of conidial heads may be seen (Fig. 1). Where direct spread to organs such as the kidneys occurs, the lesions resemble those described below.

The other types of lesions are found mainly in the lungs and their structure depends on the degree of reaction of the tissue to the infecting fungus, and this depends to some extent on the precise situation. In one type the hyphae are easily seen spreading in all directions throughout the lung tissue. The inflammatory exudate varies enormously in amount. The hyphae emerge from the alveoli and are seen ramifying within the vestibulae. It has been noticed that they may even penetrate into the parabronchi. (Fig. 2.) In some cases, however, the inflammatory exudate is great in amount and of a chronic type and all degrees of severity of the process can be seen. Often the hyphae are difficult to find, or may not be seen at all in such lesions, even when the gross appearance and the result of culture make the diagnosis certain. It might be thought, and indeed it has been stated, that the density of the inflammatory exudate hides the hyphae from view, but that this is not always so is evident from the fact that hyphae may be seen even in the vestibulae when the inflammatory exudate is most obvious. Hyphae are easy to recognise and in some cases occur only in a few groups in each

section where they stand out clearly enough. Prolonged search of serial sections might reveal them more often. It is possible that such lesions with much reaction and little infecting agent represent the later stages of the disease, or they may be the result of over-action by certain hosts. The parallel to secondary tuberculosis of man is evident, but must not be over-stressed. There is always a most intense congestion of the whole lung, however young the lesions may be.

The last type of lesion is the nodular or mycetomatous. These may occur in organs with generalised lesions as well, or they may be separated by relatively normal lung, in which cases the generalised congestion is still very marked. The earliest change is simply a small area of necrosis with surrounding inflammatory exudate. Such early lesions are often found in the very margins of vestibulae, a fact which is manifestly compatible with invasion of the lung by inhalation of spores (Fig. 3). The process is a slow one and later a typical chronic abscess is produced with a necrotic centre surrounded by recent granulation tissue and then fibrous tissue outside which lies the congested, but otherwise normal lung. In birds, where giant cells are usually absent from tuberculous lesions, the tuberculoma and the mycetoma are often difficult to distinguish from each other and from a simple chronic abscess, especially in the later stages of all three. The presence or absence of acid-fast bacilli, hyphae or endotheliod cells are of value in diagnosis. Once again, however, hyphae may be difficult to find or be prominent only in small regions near the periphery. Sometimes, however, a dense mycelium, and even conidia may be found, in the inner layers or the centre, with extremely little reaction at all on the part of the surrounding tissues.

The presence of giant cells in a mycetoma is very variable. They are found usually at the periphery and may form an almost complete ring round the lesion. In one case, the very earliest lesions found contained numbers of these cells. Usually they are of the ordinary foreign-body type, but quite often they are of a rather unusual cylindroid or " epithelioid " shape. However, it must be stressed that these granulomas are by no means the only lesions found in mycotic infections, although some of the older descriptions stressed them to the exclusion of the more widespread types.

The appearances described above would appear to be referable to differences in the invasiveness of the organism and the resistance of the host tissues. Where the fungus is highly virulent and the host not very resistant, the mycelium may spread throughout the tissues with little inflammatory reaction, whereas, with a resistant host and not so invasive an organism, the mycetoma will result. The occurrence of the colony of fungus, resembling a mycetoma but with little surrounding reaction, might be explained as the effect of some toxic product of the fungus preventing the migration of inflammatory cells to the area.

MIXED MYCOSIS AND TUBERCULOSIS

The simultaneous occurrence of the two infections was first pointed out by Scott (1930). On reading his account, it is evident that he made the diagnosis of the mixed infection when he found acid-fast bacilli in sections of lesions from birds which he would otherwise have classed as suffering from mycosis. He does not appear to have tried to culture tubercle bacilli from such cases; moreover, it is not perfectly clear whether or not he considered fungi and tubercle bacilli as acting together in the same lesion. In our experience they do not, but are present in separate lesions in different sites in the same bird, although we have collected only six cases of "mixed" infection. Moreover, in infected birds we find fungi in the respiratory system and acid-fast bacilli in the liver and spleen. The records of the Zoological Society's Prosectorium show that the two organisms have been seen in smears from the same lesion, but this has not happened in our cases. We have found nothing peculiar about the histology of either lesion in cases of mixed infection, in fact the separation between them is confirmed.

In only one case (70) were we able to cultivate avian tubercle bacilli from the lesions of "mixed" infections, and in this case the fungus isolated was *Candida albicans*. This is of particular interest in view of previous reports of anti-tuberculous antibiotics from *Aspergillus fumigatus*. Vaudremer (1912, 1913) appears to have been the first to observe the antibiotic effect of *A. fumigatus* filtrates on the tubercle bacillus: more recently, Soltys (1944) and Ashesov and Strelitz (1945) have drawn attention to the destruction *in vitro* of tubercle bacilli by filtrates from this fungus. Waksman (1947) may be consulted for information on other antibiotics produced by this organism. Of our cases of mixed infection, all had been over two years in captivity. The records of the Swinhoe's pheasant (75) are incomplete, but it had probably been in captivity for some years. Only the pochard (38) was a water bird.

EPIDEMIOLOGY

This subject has given rise to endless controversy between veterinary and comparative pathologists, aviculturists and others. The literature is far too extensive to review here, but three points are evident. Firstly, mycosis, in contrast to tuberculosis, is a disease of newly-caught wild birds, especially water birds (see Table I); secondly, it occurs in definite epizootics as well as sporadically; and thirdly, the distribution of the lesions, both on naked eye examination and often microscopically, points to invasion via the air-passages.

It is usually doubted that mycosis occurs in the wild state. However, it is certain that the king penguin may die of advanced mycosis within a few days of capture (Dr. Lillie, personal communication) and similar observations have been communicated to us

about the Manx shearwater, from which *Aspergillus fumigatus* was isolated, the Magellan jackass penguin and the blackfooted penguin. Of these birds, the first has virtually no contact with any other animal, the second has very little contact with man, but the penguins are more doubtful, considering the localities in which these observations were made. In case 21, an osprey, infection must either have been contracted in the wild or developed extremely rapidly after capture. This sea bird is very wild and shy; it is also fierce and a predator. The particular specimen examined was blown on to the deck of a ship in the North Sea, having been driven by severe storms far south of its usual haunts. It died at Regent's Park two weeks after capture, apparently from fright and refusal to feed, a not uncommon occurrence with such birds. At *post mortem*, mycotic lesions were present in the air-sacs; they were quite small and were not held responsible for death, but as far as could be judged they were not very recent.

TABLE I

TABLE TO CONTRAST THE DEATHS FROM MYCOSIS AND TUBERCULOSIS DURING 1946 AND 1947

Cause of death	*Number of birds*	
	Under 6 months in Gardens	*Over 6 months in Gardens*
Mycosis	62	14
Tuberculosis	4	36

Cases of mixed infection are not included

The two main schools of thought on the epidemiology of mycosis are that either the condition is endemic in the wild and is "unmasked" by unnatural conditions of captivity, or that it is a new hazard of captivity to which wild birds have a low resistance. Our observations would appear to support the former view, although the two are not mutually exclusive. *A. fumigatus* is a common mould of the soil and of mouldy grain and the possible implication of the latter in the aetiology of the disease in captive birds was considered by Scott (1927). Attention may also be drawn to the oft-repeated statement that the incidence of aspergillosis of man in France was particularly high among squab feeders who, presumably, contracted the infection from grain taken into their mouths. It appears never to have been pointed out that since mycosis is in marked contrast to tuberculosis as regards the period after captivity at which it arises, a different aetiology for the two conditions is implied. It is doubtful if tuberculosis is ever met with in truly wild birds, most cases reported being in subjects which may well have been in contact with domestic stock. There are many references, but the position is well summarised by Hamerton (1936).

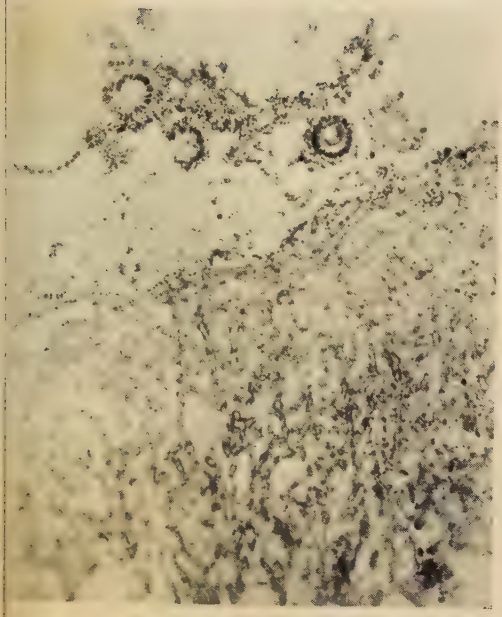

FIG. 1

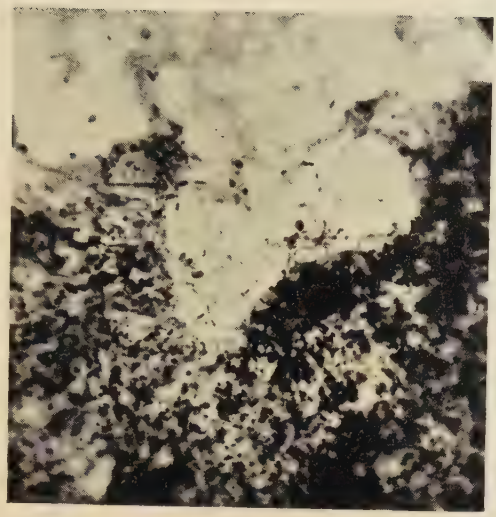

FIG. 2

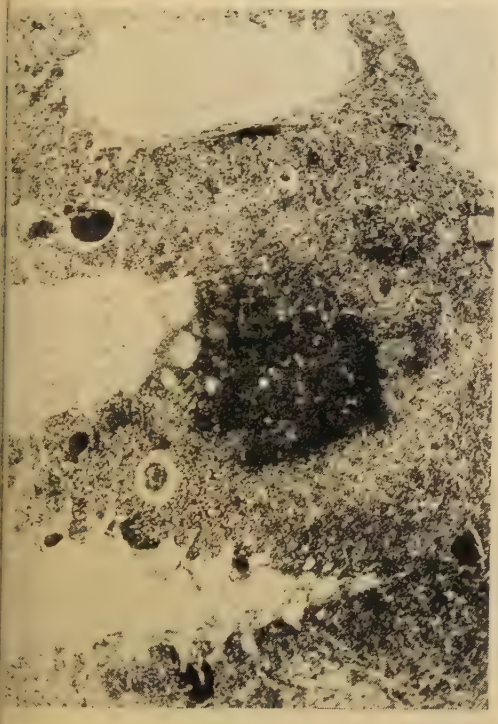

FIG. 3

FIG. 4

FIG. 1.—Case No. 42. Common guillemot. Mycelium of *Aspergillus fumigatus*, with conidial heads on the free surface, growing on the internal surface of an air-sac. Magnification = × 250. Stained haematoxylin and eosin.

FIG. 2.—Case No. 36. Blue-winged goose. Mycelium of *Aspergillus fumigatus* ramifying within the vestibulae. Magnification = × 250. Stained Heidenheim's haematoxylin.

FIG. 3.—Case No. 25. Cormorant. To show the relation of a small mycetoma to the vestibulae of a parabronchus. Magnification = × 60. Stained haematoxylin and eosin.

FIG. 4.—Case No. 68. Magellan jackass penguin. Edge of a small early mycetoma to show the extreme congestion of the surrounding lung and the non-specific nature of the inflammatory exudate. Magnification = × 250. Stained haematoxylin and eosin.

SPECIES OF BIRDS AFFECTED

In Table II the birds have been classified into orders on the system adopted by Carmichael Lowe (1929). It will be evident that several orders are heavily represented, especially as some of these, *e.g.*, Pellicaniformes and Sphenisciformes, have far fewer species than others, *e.g.*, Passeriformes, nor are they represented more frequently in Zoological collections than some of the larger orders. In our series it will be seen from Table II how great a proportion of the cases was seen in one epidemic which arose among king penguins in May, 1947. Of 24 birds admitted at that time, only five survived to the end of the year, while only two of the dead ones failed to show mycotic lesions, however small. These birds experienced bad conditions as they came to a very warm late spring and prolonged summer just when they would have expected to experience great cold.

The significance of this high incidence of mycosis among certain types of bird is obscure. It cannot be attributed to a high degree of susceptibility since the majority live in the water and some spend their time on land far from all grass or other animals, *e.g.*, the king penguin. It has been suggested, therefore, that they have a low acquired immunity to fungal spores in grass, hay or straw. This, however, does not explain the high incidence among the various types of duck.

CLINICAL COURSE

Many of the birds found to have quite advanced lesions come to necropsy with no clinical history whatsoever. Radiography of the lungs of birds in an infected flock is no help in diagnosis, as so many other structures may intervene and obscure even the cervical air-sacs. In some birds, however, signs and symptoms are recognised for some weeks before death. The most obvious are emaciation, which may be extreme, and respiratory distress, while such general signs as loss of appetite and poor plumage may be observed also. The respiratory distress may be acute with heaving movements of the carina of the sternum on inspiration, accompanied by ballooning of the cervical and interclavicular sacs in the base of the neck on expiration. In the penguins, with their upright posture, the neck may be drawn down into the body with each inspiration, presumably to give more purchase to the muscles raising the sternum.

With these signs and symptoms the diagnosis may often be made, especially in the likely species of bird and with known infection in the flock. Recent arrival in captivity and the absence of obvious lesions in the nares or fauces are other points of importance. The course is invariably downhill and no treatment is known to be of any help.

MYCOLOGY

Cultures were made at necropsy from 68 of the birds listed in Table II. Of these, 45 proved to be *Aspergillus fumigatus* Fresen.,

TABLE II

LIST OF CASES WITH RELEVANT DETAILS

No.	*Name of bird*	*Sex*	*Origin*	*Time in Zoo*	*Organism*	*Site of lesions*
	Order Passeriformes					
1.	Carrion crow, *Corvus corone*	♂	Collected	1 year	Sterile	Upper right lung
2.	White-breasted Negro finch, *Nigrita fusconata*	♂	„	10 days	*Aspergillus fumigatus*	One lesion left lung
3.	Grey-headed bristle-billed bulbul, *Bleda canicapilla*	♂	„	6 weeks	„	R.A.T., right lung and invading pericardium and upper surface of the liver
4.	Cape Palmas mannakin, *Spermestes cucullatus*	♂	„	21 months	Sterile	Left lung and both A.T.s
5.	Red-crested cardinal, *Paroaria cucullata*	♀	Presented	10 months	*A. fumigatus*	Both lungs
6.	Crimson-chested superb sunbird, *Cinnyris johnae fasciata*	♂	Collected	7 weeks	No culture	Granuloma of mouth
7.	„ „	♂	„	8 weeks	„	„
8.	Baya weaver bird, *Ploceus philippinus*	♂	Dealer	1 week	*A. fumigatus*	Abscess of lung
9.	Bengal weaver bird, *Ploceus benghalensis*	♂	„	2 weeks	No culture	„
	Order Trogoniformes					
10.	Quetzel, *Pharomacrus mocinno*	♀	Exchanged	1 month	*A. fumigatus*	Both lungs and all sacs
11.	„ „	♂	„	4 months	„	L.A.T.
	Order Coraciiformes					
12.	Kookaburra, *Dacelo gigas*	♀	Dealer	10 years	*A. fumigatus* A.F.B.	R.A.T., right lung, liver and spleen
13.	Great Indian hornbill, *Dichoceros bicornis*	♂	Collected	12 weeks	*A. fumigatus*	L.A.T. and both lungs
14.	Indian pied hornbill, *Anthacoceros malabaricus*	♀	„	4 weeks	„	L.A.T.
	Order Psittaciformes					
15.	Red-faced love bird, *Agapornis pullaria*	♂	„	1 week	No culture	Mass in mid-line involving upper parts of both lungs
16.	Grey parrot, *Psittacus erithacus*	♂	„	2 weeks	*A. flavus*	R.Ab., L.Ab. and R.T
17.	„ „	♂	„	3 weeks	Sterile	L.A.T.
18.	Budgerigar, *Melopsittacus undulatus*	♂	Born in Zoo	1 year	*A. fumigatus*	R.A.T. and lower left lung
19.	Scarlet lory, *Domicilla garula*	♂	Collected	1 week	No fungi	Both lungs and A.T.s
20.	Grey parrot	♂	Presented	1 month	No culture	Abscess of right lung
	Order Accipitriformes					
21.	Osprey, *Pandion haliaetus*	♂	Wild caught	2 weeks	*A. fumigatus*	Lower right lung
22.	Hooded vulture, *Necrosyrtes monachus*	♂	Collected	1 month	„	All sacs and pericardium
23.	West African sparrow hawk, *Accipiter melanoleucus. temmincki*	♀	„	3 months	*Candida albicans*	Left lung and both A.T.s
24.	Lammergeyer, *Gypaetus barbatus grandis*	♂	„	2 months	„	Both lungs and all sacs
	Order Pelecaniformes					
25.	Cormorant, *Phalacrocorax carbo*	♂	„	2 days	*A. fumigatus*	Both lungs and cervical sacs
26.	Gannet, *Morus bassanus*	♂	„	1 week	*A. nidulans*	Pericardium only
27.	„	♂	„	3 weeks	*A. fumigatus*	Both lungs and all sacs

TABLE II.—*Continued*

No.	*Name of bird*	*Sex*	*Origin*	*Time in Zoo*	*Organism*	*Site of lesions*
	Order Phoenicopteriformes					
28.	Chilian flamingo, *Phoenicopterus chilensis*	♂	Exchanged	1 month	*A. fumigatus*	Trachea only.
	Order Anseriformes					
29.	Eider duck, *Somateria mollissima*	♀	Purchased	1 month	*A. flavus*	Both lungs and small patch in trachea
30.	Sheld duck, *Tadorna tadorna*	♀	Presented	1 month	*C. albicans*	Lower left lung and L.A.
31.	Black-necked swan, *Cygnus melancoriphus*	♂	Presented as a cygnet	4 years	*A. fumigatus*	Pericardium, right lung R.Ab., L.Ab., L.A.T., R.A.T.
32.	Red-crested Pochard, *Netta refina*	♂	Dealer	1 month	*C. albicans*	Syrinx, both lungs, Ab. and thoracic sacs
33.	Cape teal, *Anas capensis*	♂	Exchanged	3 months	„	Both lungs and R.A.T
34.	Australian sheld duck, *Casarca tadornoides*	♀	Dealer	2 years	No fungi	Both lungs, I.S. and liver
35.	Red-crested pochard	♂	„	6 weeks	*A. fumigatus*	Both lungs, R.A.T., rt. kidney
36.	Blue-winged goose, *Cyanochen cyanopterus*	♂	„	2 years	„	Left lung
37.	„	♂	„	2 years	„	Syrinx, L.A.T., L.Ab. upper left lung
38.	Pochard, *Aythya ferina*	♂	Presented	5 years	*A. fumigatus* A.F.B.	R.P.T., cervical sacs, lungs, spleen and liver
39.	Red-crested pochard	♀	Dealer	?	*A. fumigatus*	Both lungs, C. and T. sacs, spread to abdomen
	Order Ardeiformes					
40.	Scarlet ibis, *Eudocimus ruber*	♂	Exchanged	3 months	No culture	Both lungs and all sacs
	Order Alciformes					
41.	Puffin, *Fratercula arctica*	♂	Collected	1 month	*A. fumigatus*	L.A.T., R.A.T., L.Ab, R.Ab. outer surface of gizzard
42.	Common guillemot, *Uria aalge*	♂	Found injured	2 days	„	R.Ab. and L.C.
43.	„ „	♂	Collected	11 days	„	Both lungs and A.T.s
44.	„ „	♀	„	5 weeks	No fungi	Left lung only.
45.	Puffin	♂	„	5 weeks	*A. fumigatus*	Both lungs, R.A.T., R.Ab., and both cervical sacs
46.	Common guillemot	♂	„	5 weeks	No fungi	Both lungs
47.	„ „	♂	„	1 month	*A. fumigatus*	L.A.T.
	Order Sphenisciformes					
48.	King penguin, *Aptenodytes patagonica*	♀	Collected	2 weeks	„	Both lungs and all sacs
49.	„ „	♂	„	1 month	Sterile	Both A.T.s
50.	„ „	♂	„	1 month	„	Both lungs and all sacs
51.	„ „	♀	„	5 weeks	*A. fumigatus*	Both lungs, all sacs and both kidneys
52.	Ringed penguin, *Pygoscelis antarctica*	♂	„	5 weeks	„	All sacs, esp. L.A.T.
53.	King penguin	♂	„	7 weeks	Sterile	All sacs, esp. L.A.T.
54.	Ringed penguin	♂	„	8 weeks	*A. fumigatus*	Both lungs and all sacs
55.	King penguin	♀	„	10 weeks	„	All sacs

TABLE II.—*Continued*

No.	*Name of bird*	*Sex*	*Origin*	*Time in Zoo*	*Organism*	*Site of lesions*
56.	King penguin	♂	Collected	10 weeks	*A. fumigatus*	All sacs and little in both lungs
57.	„ „	♀	„	10 weeks	No culture	Both lungs, T. and Ab. sacs
58.	„ „	♀	„	10 weeks	„	All sacs heavily involved
59.	„ „	♂	„	10 weeks	„	Both A.T.s
60.	„ „	♀	„	11 weeks	No fungi	R.A.T.
61.	„ „	♀	„	11 weeks	„	Both lungs and A.T.s
62.	„ „	♂	„	11 weeks	„	R.C.
63.	„ „	♀	„	15 weeks	No culture	Both lungs and all sacs
64.	„ „	♀	„	16 weeks	*A. fumigatus*	Both lungs and all sacs
65.	„ „	♀	„	17 weeks	„	Both lungs, C.s and A.T.s
66.	„ „	♂	„	11 months	„	Both lungs and all sacs
67.	Magellan jackass penguin, *Spheniscus magellanicus*	♀	„	7 weeks	„	Both lungs, all sacs and main bronchi
68.	„	♀	„	5 months	„	Both lungs and A.T.s
	Order Columbiformes					
69.	Tambourine dove, *Tympanistria tympanistria*	♂	Collected	3 weeks	„	Both A.T.s and invading right lung
70.	Galapagan dove, *Nesopelia g lapagoensis*	♂	Purchased	18 months	*C. albicans* *Mycobact. tuberculosis*	L.Ab., lower left lung and liver
71.	Tambourine dove	♂	Collected	27 months	*A. fumigatus*	Both lungs and A.T.s
72.	Pheasant-tailed fruit pigeon, *Macropygia phasianella*	♂	Presented	17 years	*A. fumigatus* A.F.B.	Liver and spleen
	Order Galliformes					
73.	Quail, *Coturnix coturnix*	♂	Collected	2 weeks	*A. fumigatus*	R.Ab.
74.	Impeyan pheasant, *Lophophorus impejanus*	♀	Dealer	2 weeks	„	Both Ab. s and right lung
75.	Swinhoe's pheasant, *Gennaeus swinhoii*	♂	Exchanged	?	*A. flavus* A.F.B.	Both lungs, all sacs, liver and spleen
76.	Common peafowl, *Pavo cristatus*	♂	Bred.	3 years	*A. fumigatus* A.F.B.	Both lungs, all sacs, liver and spleen
77.	Impeyan pheasant	♀	Dealer	1 week	*A. fumigatus*	R.A.T.
78.	Temminck's tragopan, *Tragopan temmincki*	♂	Presented	4 years	„	Both A.T.s

The birds are arranged in their Orders, according to the system adopted by Low (1929). Within each Order the cases are arranged chronologically. The time spent in the Gardens is approximate and given as a rough guide. For the birds which were presented, purchased from a dealer or exchanged with other Zoos it is of little value. For those collected in the field it is of more help, although some, such as the penguins, experienced a long voyage through a great variety of climates before arrival, but British birds had been only a few days in captivity before being received. In a number of cases the bodies were too decomposed for cultures to be taken or for sections to be made.

Abbreviations. A.F.B., acid-fast bacilli seen in smears or sections from the abdominal lesions, but none obtained on culture on Loewenstein-Jensen's medium; R.A.T. and L.A.T., right and left anterior thoracic air-sacs respectively; R.Ab. and L.Ab., right and left abdominal air-sacs respectively; R.C. and L.C., right and left cervical sacs respectively; I.S., interclavicular sac. R.P.T., right posterior sac.

three *A. flavus* Link. (eider duck, 29 ; grey parrot, 16 ; and Swinhoe's pheasant, 75) ; one was *A. nidulans* (Eidam) Wint. (gannet, 26). In seven cases only contaminating moulds and bacteria were found and in six *Candida albicans* was noted. Six were sterile.

To supplement these findings a series of cultures from cases of aspergillosis in birds referred to the Ministry of Agriculture's Veterinary Laboratory, Weybridge, during the first half of 1947 from widely-scattered localities in England and Wales, were examined through the kindness of Dr. R. F. Gordon and Mr. J. D. Blaxland. Only *A. fumigatus* was found in the 28 cases investigated ; eight hens, 16 chicks, one turkey, two pigeons and one Amherst pheasant. This predominance of *A. fumigatus* as the causal agent of avian aspergillosis is in line with the findings of other workers (see Lesbouyries, 1941). *A. niger* van Teigh., which was recorded as causing aspergillosis in birds in Britain by Scott (1927, 1930), was not recovered during the present survey. The pathogenic status of this species is rather uncertain.

The isolation of *A. fumigatus* calls for no special comment. The characters of this well-known species with its club-shaped conidiophores, each bearing at its apex a single series of sterigmata producing chains of conidiophores 2 to 3μ in diameter, are described in detail by Thom and Raper (1945) and will not be repeated here. There was little variation in the morphology of the various cultures (Fig. 5). Attention may be drawn to the purity of the primary cultures. *A. fumigatus* was usually the only mould, although bacterial contamination was common. The cultures from the few cases where other species of *Aspergillus* were involved were also pure, and from the necropsy and histological findings there was nothing to suggest that the more usual *A. fumigatus* was not involved.

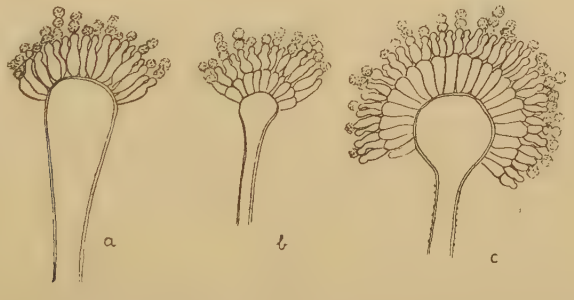

FIG. 5.—Conidial heads of (*a*) *Aspergillus fumigatus* ; (*b*) *A. nidulans* ; and (*c*) *A. flavus* in optical section. Magnification = × 500

SUMMARY

Seventy-eight cases diagnosed as aspergillosis in captive wild birds were examined in detail. The gross morbid features are tabulated. The histological features are described and discussed. Previous accounts

have stressed a granulomatous type of lesion, but a more widespread one without specific histological features appears more common. Lesions occur almost always in the respiratory system.

Cultures were made from 68 cases; 45 yielded pure growths of *Aspergillus fumigatus*, three were *A. flavus*, one was *A. nidulans*. There were no anatomical differences in the disease produced by these fungi.

The disease is essentially one of recently-captured water birds, *e.g.*, penguins, although sporadic cases do occur in birds of all types and after long periods in captivity. It is possible that it exists in the wild state far from contact with other animals. The epidemiology is discussed briefly.

ACKNOWLEDGMENTS

While the investigation was in progress, one of us (G. C. A.) was working at the London School of Hygiene and Tropical Medicine in the Department of Medical Mycology. We have to thank Dr. J. T. Duncan and the authorities of that institution for the facilities provided. The cases are published by permission of the Zoological Society of London. The photographic illustrations are by Messrs. Ilford, Ltd.

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STUDIES ON *STR. AGALACTIAE* INFECTION

VI OBSERVATIONS ON EPIZOOTIOLOGY AND THE USE OF SULPHONE* AND PENICILLIN AS AIDS TO ERADICATION†

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INTRODUCTION

It would obviously be impossible to prevent mastitis associated with *Str. agalactiae* by the usual sanitary measures, if it were an ubiquitous organism like *Bact. coli*, present in the faeces, tonsils and vagina of normal animals. Early workers on mastitis, almost certainly caused by *Str. agalactiae*, regarded the condition as an infectious disease (Nocard and Mollereau, 1887 ; Bang, 1889). More recently, Minett, Stableforth and Edwards (1933) emphasised the infectious nature of mastitis associated with *Str. agalactiae* and were the first to establish and maintain a herd free from infection by the segregation and elimination of infected animals. Further work on the same lines has been described by Little (1940), and by Plastridge *et al.* (1942), who studied 74 herds in which the initial infection rate was 40·9 per cent. They eradicated infection from 24 of these herds by the elimination of infected animals and their replacement by heifers. Despite these results, there was considerable scepticism about the possibility of eradicating infection from a herd when the work reported in this paper was begun (Minett, 1939 ; Munch-Petersen, *et al.*, 1940).

Following an extensive survey of the incidence of *Str. agalactiae* infection in this country, it was concluded that the few successful attempts to eradicate the organism were more surprising than the failures (Report, 1944), although the theoretical possibility of eradication was admitted. The last view is supported by the earlier successful attempts at eradication mentioned above and by the findings of Little (1940) and Hastings *et al.* (1943), who established and maintained herds free from *Str. agalactiae* infection, using exactly the same methods which were unsuccessful in the hands of Munch-Petersen *et al.* (1940). *Str. agalactiae* has, however, been isolated from heifer's milk before, or just after, calving, under conditions which precluded recent infection (see Minett, 1939 ; Mattick *et al.*, 1941 ; Stewart, 1943 ; Miller and Heishman, 1944).

Francis (1941) carried out a bacteriological examination of the tonsils and vaginas of cattle. He failed to isolate *Str. agalactiae*, but did isolate all the other organisms usually associated with mastitis.

* This abbreviation is used for 4 : 4′ diaminodiphenyl sulphone.

† The results obtained in this study were briefly reported by Francis (1948a).

Other workers (Harrison, 1942 ; Report, 1944) also failed to isolate *Str. agalactiae* from these sources or from faeces, although Munch-Petersen *et al.* (1940) isolated *Str. agalactiae* from the faeces on seven occasions. The above findings supported the view that much the most important source of *Str. agalactiae* infection was the udder, and showed that other organisms associated with mastitis were frequently present in the tonsil or vagina. It seemed, therefore, that there was justification for regarding mastitis associated with *Str. agalactiae*, but not with other organisms, as an infectious disease which could be eradicated. The earlier work on the Continent and elsewhere in which the acridines were used as aids to the eradication of *Str. agalactiae* infection was reviewed by Little and Plastridge (1946), and it need only be said here that the herds were not entirely freed from infection. Much work has been published on the treatment, but not on the control, of *Str. agalactiae* infection with penicillin. Johnson and Fincher (1946) did, however, greatly reduce the incidence of *Str. agalactiae* infection in two badly infected herds, although they did not eradicate the organism. Murnane (1946) described the results of treating clinical mastitis for a period of 12 months with penicillin and mentions the difficulty of eliminating or even appreciably reducing the incidence of mastitis ; Arthur (1947) expressed similar views. Edwards *et al.* (1947) failed with repeated injections of sulphanilamide to eradicate *Str. agalactiae* infection from an experimental herd.

Francis, Peters and Davies (1947a) found that sulphone was more potent than any of the sulphonamides against *Str. agalactiae* infection in the chick-embryo and mouse, and that it was more potent than its soluble acetaldehyde bisulphite derivative (Francis, Peters and Davies, 1947b). When 10 gm., suspended in liquid paraffin, are injected into one or more quarters of a lactating cow, milk levels remain above 10 mgm. per cent. for three days, and blood levels at 1 to 2 mgm. per cent. (Francis, 1947) ; in dry cows high "milk levels" are maintained for a much longer period and blood levels above 2 mgm. per cent. for five days or longer.

The foregoing facts indicated that sulphone was worthy of trial for the treatment of *Str. agalactiae* infection, and two lines of investigation were begun. Mr. J. Berger, M.R.C.V.S., treated clinical cases of mastitis encountered in practice (Berger and Francis, 1949), and we took over from him a number of herds in which mastitis had been persistent for several years, despite treatment with sulphanilamide and acriflavine. Work in these herds, in which an attempt was made to eradicate infection largely by the use of chemotherapeutic measures, is described in this paper.

TECHNIQUE

Treatment.—In the first herd which was studied a variety of treatments was used in an attempt to eliminate *Str. agalactiae* infection, but after a short time 10 to 20 gm. of sulphone became the

standard treatment and it was later replaced by 10 gm. of sulphone plus 50,000 units of penicillin. Lactating cows were usually not treated until they were giving less than two gallons of milk a day; when this stage had been reached the quarter was infused and the infusion left in for three days without milking. If this procedure caused discomfort a little milk was withdrawn. In dry cows the quarters were infused and left until the cow calved.

Bacteriological Examination.—When taking milk samples, the udder was washed with chloros solution, the ends of the teats wiped with cotton-wool soaked in alcohol and " strict " foremilk samples taken from individual quarters into bottles containing 1 ml. of glucose-saline to give a final concentration of 1 per cent. glucose. Each sample was plated on half a blood-agar plate with a loop which delivered 1/100 ml. of milk. Samples were then incubated and again streaked out on blood-agar plates. Results recording the number of organisms on the plate inoculated with 0·01 ml. of unincubated milk were indicated in the following way: (1) Numerous organisms; (2) moderate number organisms; (3) few organisms; (4) organisms on plate inoculated with the incubated sample of milk.

This technique is economical of labour; it gives an indication of the severity of infection and examination of the incubated sample detects very small numbers of organisms. In fact, the value of examining incubated milk samples has been questioned by some workers who consider that when *Str. agalactiae* can only be detected in this way there is no true " infection " of the quarters. The subject has been discussed by Francis (1948b), who presented evidence supporting the value of examining incubated milk samples. The results of examining udder tissue after death are reported later in this paper and they show that even when there is infection of this tissue there may be very few or no organisms in the milk over considerable periods of time, thus indicating that the most delicate method of diagnosis should be used. On the other hand, it is obvious that in a heavily infected herd positive results from the incubated sample may merely indicate contamination or colonisation of the teat sphincter, and the present study showed that results were more regular when a quarter was heavily infected than when it was lightly infected: the same would be true in the study of any other infection. The behaviour of the different grades of infection can be studied in the tables, but to avoid too much complication only the total results are discussed in the text.

RESULTS

Herd 1.—Herd 1 contained 14 cows, six of which had frequently shown symptoms of mastitis during the past year and had been dosed with sulphanilamide.

All cows were lactating at the first examination, when 12 quarters of six cows were found to be infected with *Str. agalactiae.* Milking was by machine, and no alteration was made in the posi-

tion of the cows or the stable hygiene. Frequent examinations were made, and it can be seen from Fig. 1 that the cumulative total of infections rose rapidly due to the fact that quarters were frequently " becoming infected " and recovering from infection.* At the third herd test (three weeks), 18 quarters were infected, and it is obvious that during this early period the cowshed was a heavily infected environment and there was constant transfer of infection from teat to teat, together with apparent recovery from infection. Following the third test, infected cows were milked last and this, combined with the successful treatment of some of the most heavily infected quarters, caused a steady drop in the number of infected quarters until by the seventh herd test (nine weeks) only six quarters were infected. The low figure at the seventh test was partly due to temporary recoveries following experimental therapies. By the time of the 19th examination (41 weeks), 13 quarters had been successfully treated and the herd was entirely free from infection and remained so for five more tests, up to 56 weeks.

At the 25th examination (61 weeks) a purchased cow was found infected in all quarters. During the next 40 weeks, five infected quarters were found in purchased cows. Just before the 28th examination (107 weeks), seven first and two second calf cows were added to the herd, but only one quarter was found infected in a second calf cow of this group. All these infections in purchased cows were treated successfully, and during this period only one quarter was found lightly infected in one of the original cows. This was also treated successfully.

At the last examination (178 weeks), 17 cows were sampled and all were free from infection, but only two of the original 14 cows were still in the herd. At 61 weeks, when the herd had been free from *Str. agalactiae* infection for 15 weeks, it contained ten (71·4 per cent.) reactors to the tuberculin test. Tuberculosis was eradicated during the subsequent 40 weeks, which accounts for the large numbers of disposals and purchases which took place during this period and for the reintroduction of *Str. agalactiae* infection.

The main facts about the control of infection in Herd 1 are summarised in Table I. The calculated permanent natural recovery rate (Table I) was 15·4 per cent., whereas the permanent recovery rate following all forms of treatment was 64·7 per cent. These results are more significant when one realises that the average severity of infection (see page 227 and Table III) in quarters recovering without

* It is realised that *Str. agalactiae* is not necessarily always isolated from an infected quarter, but for the purpose of this paper a quarter is described as uninfected if *Str. agalactiae* was not isolated at any given examination and infected if *Str. agalactiae* was isolated. The term " natural recovery " is used to describe the non-isolation of *Str. agalactiae* when *Str. agalactiae* had been isolated from a quarter at the preceding test, the reverse sequence of events is taken to indicate that the quarter had " become infected." On the other hand, the use of the term " recovery following treatment," means that the quarter was free from infection at all subsequent tests.

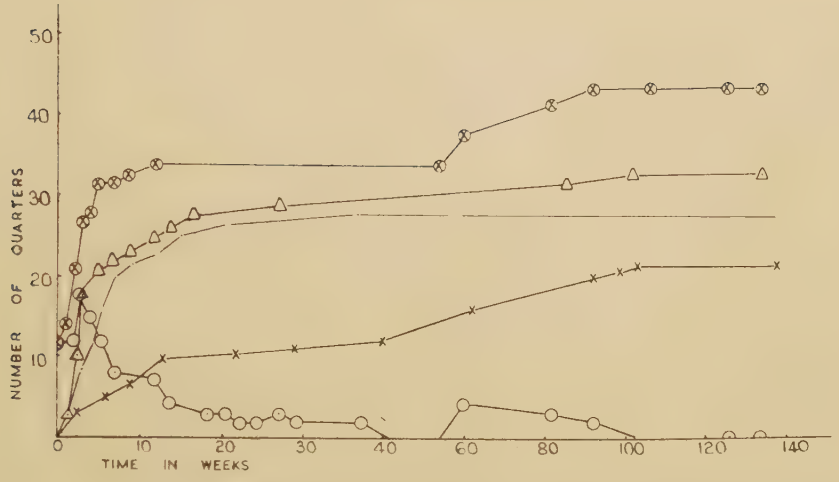

FIG. 1.—The behaviour of *Str. agalactiae* infection in Herd 1.

After 60 weeks the " total of infected quarters " increased without a corresponding increase in the " quarters becoming infected " ; the increase is due to the purchase of infected cows. The time scale is halved after 40 weeks.

Keys to Figs. 1 *and* 2 :

⊙ Number of infected quarters.
⊗ Cumulative total of infected quarters.
Δ „ „ quarters becoming infected.
· „ „ „ recovering naturally.
× „ „ „ „ with treatment.

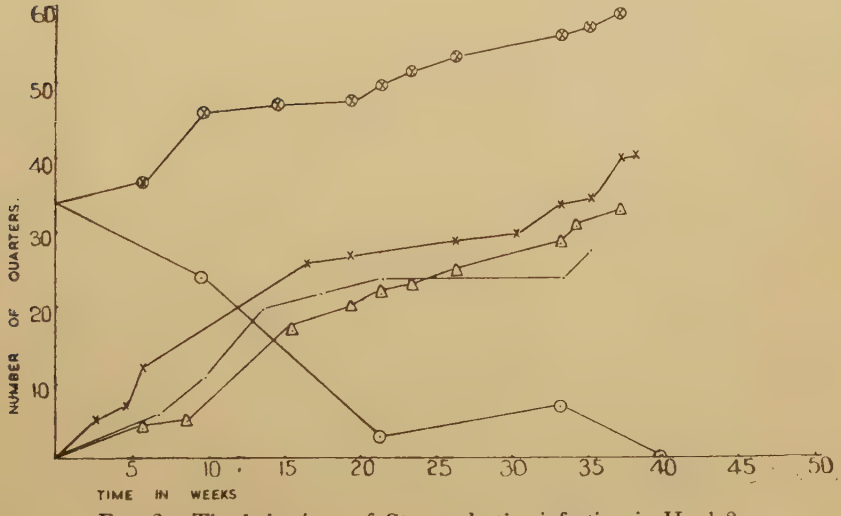

FIG. 2.—The behaviour of *Str. agalactiae* infection in Herd 2.

treatment was 1·34 and the duration of infection 1·25 weeks. In quarters recovering after treatment the average severity of infection was 2·57 and the period of infection 9·37 weeks.

Herd 2.—There had been persistent trouble from mastitis in this herd for three years despite the use of sulphanilamide and of acriflavine infusions. At the first examination 50 quarters and 26 of 52 cows were infected, but for various reasons five cows with 15 infected quarters were considered unprofitable by the owner and sold; these five cows are not considered further or included in the

TABLE I

SUMMARY OF EPIZOOTIOLOGICAL DATA IN HERDS 1 AND 2

No. of cows at first herd test	*No. added to herd*		*No. of cows at last herd test*	*No. infected at first herd test*		*No. of infections in purchased cows*		*No. becoming infected*		*Total No. of infections*		*No. of infections recovering naturally and % permanent recovery rate*	*No. of infections recovering following treatment and % permanent recovery rate*	*No. incurable*
	Cows	*Heifers*		*Cows*	*Quarters*	*Cows*	*Quarters*	*Cows*	*Quarters*	*Cows*	*Quarters*			
Herd 1														
14	9	12	17	6 42·9%	12 21·4%	2	5	9	33[4]	17	44[1]	27[3 5] 15·4%	22 64·7%	1
Herd 2														
47	8	5	41	21 44·7%	35 18·6%	2	3	3	33[4]	27	60[2]	28[3 5] 4·5%	41 89·1%	1

[1] Sixteen were positive at a single examination only, and 17 were never positive by direct plating; six quarters became re-infected after apparent recovery, giving a total of 50 "infections."

[2] Eighteen were positive at a single examination only, and 15 were never positive by direct plating; eleven quarters became re-infected after apparent recovery, giving a total of 71 "infections."

[3] An inspection of this table gives the impression that natural recovery occurred very frequently. This is true, but an examination of the text and of Table II shows that the permanent natural recovery rate was only 10·9 per cent., whereas that following all forms of treatment was 78·7 per cent. Table III also shows that the quarters recovering naturally were much more lightly infected than those recovering following treatment. There can be little doubt that most of the "infections" which recovered naturally were little more than contaminations of the teat canal in a heavily infected environment. On the other hand, a quarter may be found infected at post-mortem examination, when few or no organisms have been detected in the milk.

[4] In the two herds, 17 quarters which had apparently recovered became re-infected; 14 "reinfections" and 25 new infections occurred in cows with other infected quarters.

[5] In the two herds, 34 of these quarters were positive at a single examination only, and 32 were never positive by direct plating.

TABLE II

RECOVERY RATES FROM *Str. agalactiae* INFECTION

Infections detected	*Herd 1*		*Herd 2*		*Total*		
	By direct[1] plating	*After incubation of sample*	*By direct plating*	*After incubation of sample*	*By direct plating*	*After incubation of sample*	*Total*
Calculated permanent natural recovery rate	5/80 6·3%	13/37 35·1%	0/50 —	3/22 13·6%	5/130 3·8%	16/62 25·8%	21/192[2] 10·9%
Permanent recovery after all forms of treatment	17/28 60·7%	5/6 83·3%	36/41 87·8%	5/5 100%	53/69 78·6%	10/11 90·9%	63/80 78·7%

[1] See page 227 for explanation of technique.

[2] Any quarter infected at one test could " recover " at the next test and remain free from infection. There were 192 occasions when this could have occurred. In fact, it only occurred 21 times, and many of the quarters which did recover had only been infected at a single examination.

TABLE III

SEVERITY AND DURATION OF INFECTION IN QUARTERS RECOVERING PERMANENTLY IN HERDS 1 AND 2

		Number of quarters	*Average period of infection weeks* [1]	*Average number of examinations of each quarter* [2]	*Average number of positive examinations of each quarter* [2]	*Average severity of infection* [3]
Quarters recovering naturally	Herd 1	21	1·25	2·6	1·6	1·34
	Herd 2	19	0·65	1·15	1·15	1·45
Quarters recovering with treatment	Herd 1	22	9·37 [3]	6·1	5·0	2·57
	Herd 2	41	10·1 [3]	3·3	2·8	2·3

[1] If a quarter was positive on only one occasion the period of infection was recorded as " 0 " weeks.

[2] Figures in these columns apply to the period up to the last positive examination.

[3] The exact difference in the number of organisms present when a quarter was identified as 4 or 1 cannot be given, but it would be at least 500 and often much greater.

[4] Most of these quarters were already infected at the first examination of the herd, so the actual period of infection was longer than that shown.

tables. The order of the cows was changed a fortnight after the first examination so that uninfected cows were milked first and, in general, the most severely affected cows milked last. The milking machines were always sterilised after the morning milking and, although sterilisation after the evening milking was advised, its efficiency, particularly during the earlier part of the work, was uncertain.

The subsequent course of events is shown in Fig. 2 and summarised in Table I. By the fifth herd test (40 weeks) 36 quarters had recovered following treatment and the herd was free from infection at the sixth herd test (56 weeks); after this it was left without examination for ten months, except that three purchased cows were tested. A test carried out at the end of this period did not reveal *Str. agalactiae*, nor was it isolated at the final examination 20 weeks later.

The calculated permanent natural recovery rate (Table I) was 4·2 per cent., whereas the permanent recovery rate following all forms of treatment was 89·1 per cent. These results are more significant when one realises that the average severity of infection (see page 227 and Table III) in quarters recovering without treatment was 1·45 and the period of infection 0·65 weeks, whereas in those recovering following treatment the average severity of infection was 2·3 and the period of infection 10·1 weeks.

Herd 3.—This was a herd of 50 cows in which sporadic cases of mastitis had occurred during the past two years. At the first examination six quarters of four lactating cows were found infected. Five of these quarters, together with two others, were treated successfully but one quarter in cow 202 proved highly resistant to therapy. During her second dry period, about 80 weeks after the first herd test, the quarter received three injections of 10 gm. of sulphone plus 100,000 units of penicillin, but was still infected at the fourth herd test (87 weeks) and the cow was sold. At the fifth herd test (91 weeks) *Str. agalactiae* was not isolated. The herd was again free from infection at the sixth herd test (130 weeks).

Herd 4.—In this herd of 20 cows in which there had been serious trouble from mastitis for the past year, 14 quarters of eight cows were infected at the first examination. All infected cows were lactating, but the 14 quarters were injected with 10 gm. of sulphone and 100,000 units of penicillin. The treated quarters were not milked for three days. This herd was at Bedford and frequent examinations could not be made, but a test carried out three months later showed that only one cow had an infected quarter and she was sold.

Herd 5.—Of the 35 cows in this herd three had given clots for several months. At the first examination two of these cows were found infected with *Str. agalactiae* in the four quarters which had given clots. The quarter in the other cow which had given clots was heavily infected with haemolytic staphylococci. Three other cows were found infected with *Str. agalactiae* in one quarter each. None of the infected cows was giving more than two gallons of milk, so all infected quarters were injected with 10 gm. of sulphone and 50,000 units of penicillin, which were left in for three days without milking. The milk yield was recorded once a week and treatment had no adverse effect on the yield. All cows in the herd were examined at intervals of 8, 20, 32 and 77 weeks, following treatment of the infected cows, and *Str. agalactiae* was not isolated.

The main facts concerning the eradication of infection from Herds 1, 2, 3, 4 and 5 are presented in Table IV.

Herd 6.—This was a small herd of cows kept at the laboratory. Three in-calf cows, two of which were lactating, and two in-calf heifers, were purchased from an attested and abortion-free farm. Five months after they arrived the milk was examined bacteriologically, and this was continued at frequent intervals.

Cow 1 was examined three times within ten days, and at the third examination *Str. agalactiae* was isolated by direct plating from one quarter. This quarter was not treated but was negative at three subsequent examinations, after which a series of experiments on the behaviour of the acetaldehyde bisulphite derivative of sulphone was begun. *Str. agalactiae* was not recovered at 24 examinations over a period of 62 weeks, but when the cow was going dry *Str. agalactiae* was isolated by direct plating on each of two occasions from three quarters. These quarters were injected with 10 gm. of sulphone plus 50,000 units of penicillin which were left in the udder until the cow calved. *Str. agalactiae* was not isolated at any of four subsequent examinations over a period of 30 weeks after calving nor during the subsequent dry period.

TABLE IV
CONTROL OF *Str. agalactiae* INFECTION IN FIVE HERDS [1]

Herd	*No of cows in herd at first test*	*Infection at first herd test*				*Quarters recovering after all forms of treatment*					*Weeks free from infection*
		Cows		*Quarters*							
		No.	%	*No.*	%	*No.*	%	*Average No. of examinations following successful treatment*	*No. of quarters incurable and sold*	*Weeks to eradicate infection*	
1	14	6	42·86	12	21·43	22	64·7	14·7	1	41/32[2]	15/90 (4 herd tests)
2	47	21	44·68	35	18·61	41	89·1	5·3	1	46	70 (4 herd tests).
3	50	4	8·0	6	3·0	7	63·6	3·1	1	91	40 (2 herd tests).
4	20	8	40·0	14	17·5	13	92·9	1·0	1	3	Further tests impossible.
5	35	5	14·29	7	5·0	7	100·0	3·1	0	3	77 (4 herd tests).
Total	166	44	26·5	74	11·0	86	76·8	6·8	4		

[1] In Herds 1 and 2 the epizootiology of the infection was being studied and effective measures of control were not immediately applied.

[2] Herd 1 was free from infection for the 15 weeks, commencing 41 weeks after the first examination, but for the next 32 weeks infected cows were introduced after which the herd was again free from infection and remained so for 90 weeks.

Cow 3 was examined 28 times over a period of 100 weeks. *Str. agalactiae* was then isolated from two quarters at the same time as it occurred in three quarters of Cow 1. These quarters were treated with an experimental therapy and were free from infection at three subsequent examinations. Cows 2, 4 and 5 were examined 20 to 37 times over periods of 110 to 144 weeks without isolating *Str. agalactiae*. Three home-bred heifers calved and each was examined on four occasions without detecting *Str. agalactiae*.

Quarters Affected and Average Number Affected per Cow

It can be seen from Table I that over the whole period of observation in Herd 1, 17 cows were infected in an average of 2·6 quarters each, and in Herd 2, 27 cows were infected in an average of 2·2 quarters each ; the average number of quarters affected at any one examination would be lower. The right hind quarter was most frequently (34 times) and the left fore least frequently (23 times) affected.

Influence of Str. agalactiae *Infection on the Quality of Milk*

The *p*H of all milk samples from lactating cows was electrometrically determined whenever Herds 1 and 2 were tested. The average *p*H of milk from infected quarters, compared with the *p*H of uninfected quarters on the same cows, is shown in Table V ; quarters were classified as uninfected if a recognised pathogen was not isolated at the time of the test. It will be seen that in both herds

TABLE V

EFFECT OF *Str. agalactiae* INFECTION ON THE QUALITY OF MILK

		Severity of infection with Str. agalactiae[1]				*Uninfected quarters on the same cows*[3]
		4	3	2	1	
Herd 1	*p*H	6·99	6·97	6·94	6·87	6·83
	Number of quarters	34	17	19	60	161
Herd 2	*p*H	6·94	6·93	6·9	6·88	6·71
	Number of quarters	13	6	29	29	90
Herd 1	Cells[2]	3·7	2·4	2·8	1·1	0·98
	Number of quarters	24	17	28	42	178

[1] For explanation of technique, see page 227.

[2] The number of cells was assessed and given values of 0–5. Smears given a value of 0 would have less than 1,000 cells per c.c. ; those given a value of 5, over 10,000,000 cells per c.c.

[3] Quarters containing recognised pathogens other than *Str. agalactiae* were excluded.

there was a slight but steady increase in *p*H, with increasing severity of infection. At the same herd tests in Herd 2 the average *p*H of milk from all quarters in the 20 cows which were never infected with *Str. agalactiae*, except those containing other recognised pathogens, was 6·80, which compares with a *p*H of 6·83 in the uninfected quarters of the infected cows. The figure for the uninfected group of cows is not so strictly comparable with the figures shown in Table V, as they are with each other, but it provides no evidence that there was any primary factor causing udder disturbance in the infected group of cows before invasion by *Str. agalactiae*.

In Herd 1 samples of incubated milk were examined microscopically to estimate the accuracy of this method of diagnosis (Francis, 1948b). At the same time the number of cells present was assessed and given arbitrary values from 0 to 5. Again it will be seen that, with one minor exception, there was an increase of cells with increasing severity of infection.

No detailed record was kept of the number of times that clots appeared in the foremilk, but the beneficial effect of recovery from *Str. agalactiae* infection was evident throughout the investigation. Following eradication of infection in Herd 1, clots in the foremilk were an extreme rarity instead of being very common, and when the cows in Herd 2 were divided into infected and uninfected portions clots were frequently observed in the former but rarely in the latter. As more and more cows were cured so the number of cows giving clots in the milk was reduced until, as in Herd 1, clots in the milk were a rarity and remained so for the rest of the observation period.

Examination of Udder Tissue after Death

On a few occasions it was possible to carry out a bacteriological examination of the udder tissue of cows after slaughter. The udder was divided into right and left halves and each half cut horizontally in three planes. The cut surfaces of the tissue were seared and three samples of tissues were then taken from the upper, middle and lower planes of each quarter, giving a total of nine samples per quarter. The portions of tissue were incubated for 24 hours in glucose broth and the cultures streaked on blood-agar plates.

Five cows, whose milk had been examined on numerous occasions, were examined in this way. The fact that *Str. agalactiae* was not isolated from the tissue of three quarters which had been heavily infected with *Str. agalactiae* before treatment with sulphone provides further evidence that these quarters were completely freed from infection. On the other hand, the isolation of salicin-fermenting strains of *Str. agalactiae* from the tissue of three quarters* of cow 6 indicated that treatment with sulphone, and later penicillin, had

* The original strain isolated from these three quarters fermented salicin, but two quarters were injected with a non-salicin-fermenting strain three weeks before slaughter. The non-salicin-fermenting strain was also recovered from the two injected quarters but from no others.

failed to eradicate infection, although *Str. agalactiae* had not been isolated from the milk of these quarters for about one and a half years. *Str. uberis* was isolated from the tissue of four quarters, the milk from which had never yielded *Str. uberis* despite numerous examinations of incubated milk samples. *Str. dysgalactiae* was isolated twice from the udder tissue of two quarters in similar circumstances, and *Str. bovis* from four quarters. On the other hand, the results in

TABLE VI

THE PROPORTION OF QUARTERS RECOVERING BACTERIOLOGICALLY FOLLOWING THE INJECTION OF 10 GM. OF SULPHONE, OR 10 GM. OF SULPHONE PLUS 50,000 UNITS OF PENICILLIN[1]

		Dry		*Lactating*		*Dry and lactating*	
	Herd	*Number of quarters*	*% Recovery*	*Number of quarters*	*% Recovery*	*Number of quarters*	*% Recovery*
Sulphone	1[2]	12/15	80·0	3/7	42·9	15/22	68·2
	2[3]	13/13	100·0	8/11	72·7	21/24	87·5
	3	1/2	50·0	4/6	66·7	5/8	62·5
	Total	26/30	86·7	15/24	62·5	41/54	75·9
	Berger & Francis, 1949	24/33	72·7	5/9	55·5	29/42	69·0
	Total	50/63	79·4	20/33	60·6	70/96	72·9
Sulphone/Penicillin	1			2/2	100·0	2/2	100·0
	2	4/4	100·0	15/17	88·2	19/21	90·5
	3	0/1	—	2/3	66·7	2/4	50·0
	4			13/14	92·9	13/14	92·9
	5			7/7	100·0	7/7	100·0
	6	3/3	100·0			3/3	100·0
	Total	7/8	87·5	39/43	90·7	46/51	90·2
	Berger & Francis, 1949	52/54	96·3	48/60	80·0	100/114	87·7
	Total	59/62	95·2	87/103	84·5	146/165	88·5
Total—both treatments		109/125	87·2	107/136	78·7	216/261	82·8

Total recovery of all quarters classified according to severity of infection[4]

	4	3	2	1[4]	Total
Number of quarters ...	62/79	37/47	66/77	50/58	216/261
Per cent recovery	78·5	78·7	85·7	86·2	82·8

[1] A few quarters were injected with 20 gm. of sulphone and a few with 10 gm. of sulphone, plus 100,000 units of penicillin.

[2] In Herd 1, one quarter recovered following treatment with the acetaldehyde bisulphite derivative of sulphone, and four following the injection of 100–300 thousand units of penicillin as single injections.

[3] One quarter in Herd 2 recovered following an experimental therapy.

[4] See page 227 for explanation of technique.

TABLE VII

RESULTS FOLLOWING VARIOUS METHODS OF TREATING *Str. Agalactiae* INFECTION

Author	*Treatment*	*Number of quarters*		
		Treated	*Recovered*	
	Over 2 doses of penicillin			%
Byrne *et al.*, 1946	10–100,000 units × 3	179	142	79
Hardenbrook *et al.*, 1946	25,000 units	44	37	84
Murnane, 1946	25,000 units + 5,000 units × 2	14	10	71
Murphy & Pfau, 1945	10–20,000 units × 5	42	38	91
Report, 1946	10–20,000 units × 4	300	246	82
Schalm & Casselberry, 1946	10–25,000 units × 4–5	145	116	80
Spencer, *et al.*, 1947	50,000 units × 3–4	75	66	88
Watts, 1947	10–20,000 units × 4	75	67	89
Total		960	790	82·3
	Two doses of penicillin			
Byrne *et al.*, 1946	10–100,000 units	179	111	62
Hardenbrook *et al.*, 1946	25,000 units	44	34	77
Murnane, 1945	15,000 units	57	41	62
Report, 1946	20,000 units	154	123	80
Watts, 1947	20–40,000 units	333	248	74
Total		769	557	72·4
	A single dose of penicillin			
Baker, 1945	25,000 units	32	16	50
Byrne *et al.*, 1946	10–100,000 units	179	62	34
Hardenbrook *et al.*, 1946	25,000 units	44	27	61
Murnane, 1945	15,000 units	24	9	37
Murnane, 1946	100,000 units	21	8	38
Murphy & Pfau, 1945	5,000–200,000 units	15	5	33
Report, 1946	100,000 units	140	81	58
Spencer, *et al.*, 1947	50,000 units	225	68	28
Watts, 1947	100,000	40	29	72
Total		761	326	42·8
Edwards & Brownlee, 1946	15 gm. of sulphanilamide × 4	105	78	74
Edwards *et al.*, 1947		24	17	71
Total		129	95	73·6
This study	10–20 gm. of sulphone × 1[1]	96	70	72·9
„	10 gm. of sulphone + 50,000 units of penicillin × 1[2]	165	146	88·5
Total		261	216	82·8

[1] A few quarters were treated with 20 gm. of sulphone.

[2] A few quarters were treated with 10 gm. of sulphone + 100,000 units of penicillin.

cow 11 indicate that apparently heavy infections with haemolytic staphylococci or *Str. dysgalactiae* may become completely eliminated without treatment. The examination of two quarters of cow 26 indicated that the injection of 30,000 or 3,000 organisms had failed to establish infection with *Str. agalactiae*. The injection of 3,000,000 or 300,000 organisms into two other quarters of cow 26 did, apparently, establish infection of the tissues, although the organism was not isolated from the milk before slaughter. With the injection of 30,000,000 organisms into two quarters of cow 6, *Str. agalactiae* was isolated both from the milk and the tissue, but it was very evident in both cows that milk in the udder did not behave as an ordinary culture medium for *Str. agalactiae*.

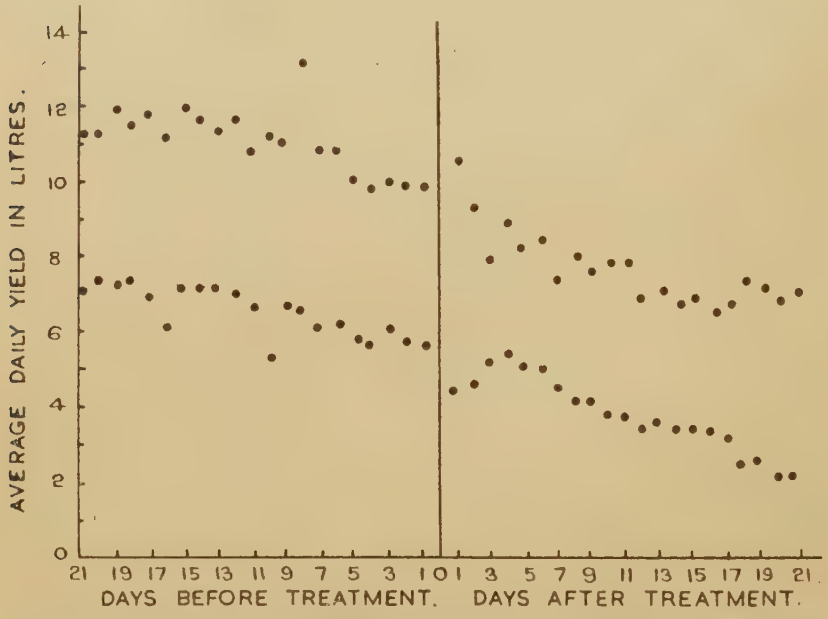

FIG. 3.—Average daily milk yield of eight cows (bottom line) 21 days before and 21 days after the injection of 10 gm. of sulphone and 100,000 units of penicillin (which were left in for three days) into 14 quarters, and of 12 untreated cows in the same herd over the same period (top line).

Efficiency of Treatment

The results of treatment with sulphone in oil, alone or in combination with penicillin, are shown in Table VI, together with the results obtained by Berger and Francis (1948). The average results are compared with those obtained with penicillin by other workers in Table VII. In both dry and lactating quarters a single injection of sulphone produced better results than those obtained with a single dose of penicillin. In lactating cows the results were worse and in dry cows better than those produced by two doses of peni-

cillin. With a single injection of 10 gm. of sulphone and 100,000 units of penicillin, the results in lactating cows appeared as good and in dry cows better than those obtained with multiple doses of penicillin.

An inspection of Table VII indicates that there was little correlation between the severity of infection and the recovery rate following treatment; this was rather surprising in view of the fact that natural recovery occurred more commonly in lightly than heavily

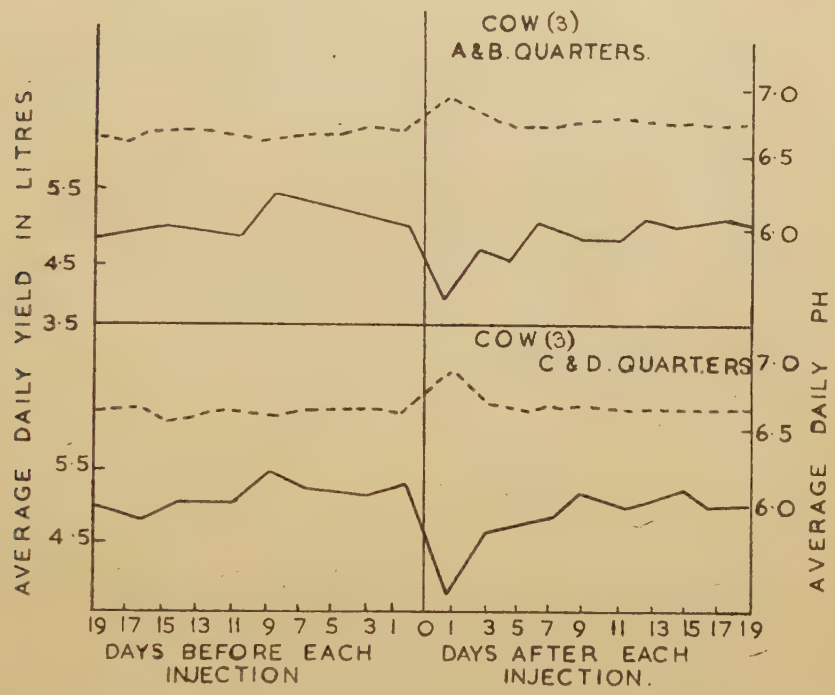

FIG. 4.—Average yield and *p*H of milk for 19 days before and 19 days after each injection of various sulphonamides into the udder of cow 3. The two left quarters (A and B) were injected with 10 gm. of sulphone on 17.10.44; 2 gm. of sulphone as the acetaldehyde bisulphite derivative on 13.11.44; 10 gm. of p'amino-benzoic acid on 12.12.44; 100 c.c. of sulphanilamide L.S.F. 10 per cent. solution on 25.1.45; 5 gm. of sulphanilamide on 22.2.45; and 5 gm. of sulphone as the acetaldehyde bisulphite derivative on 9.4.45.

Yield is given in litres (continuous line); the dotted line is the *p*H.

infected quarters. In all 81·7 per cent. of 203 quarters positive by direct plating recovered and 86·2 per cent. of 58 positive only after incubation of the sample. Watts (1947), but not Hardenbrook *et al.* (1946), or Murphy and Pfau (1945), did demonstrate a correlation between the recovery rate and severity of infection. Dry quarters did respond better than lactating, but the difference was not so great as might have been expected.

Influence of Treatment on the Quantity and Quality of Milk

The effect of treatment on milk yields arises chiefly when dealing with subacute mastitis or latent infection with *Str. agalactiae*. Daily milk recording was carried out in Herd 4, so information on this subject was obtained. Fourteen quarters of eight cows were treated with an injection of 10 gm. of sulphone plus 100,000 units of penicillin and the injection left in for three days. The milk yield of the treated cows was considerably lower than that of the 12 non-treated,

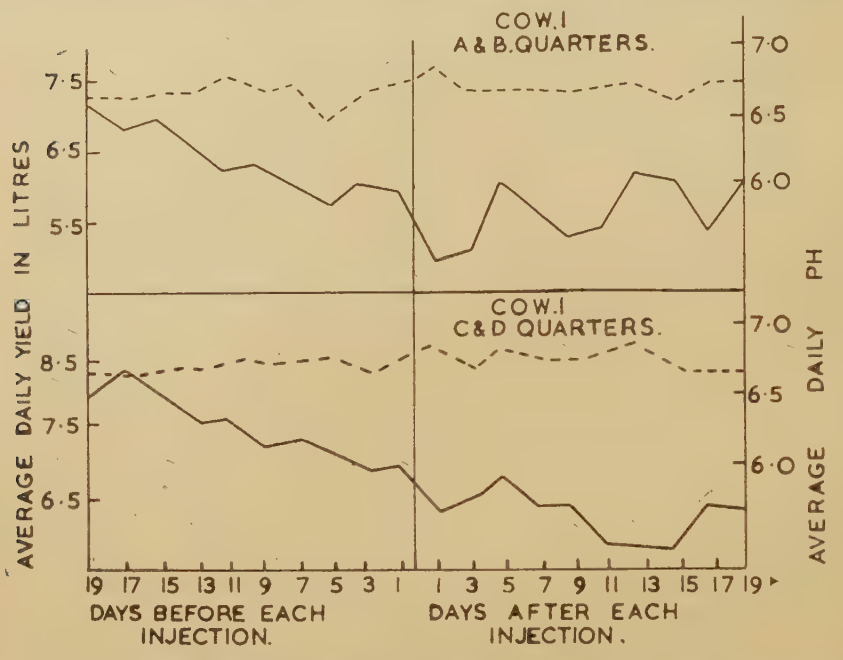

FIG. 5.—Average daily yield and *p*H of milk for 19 days before and 19 days after each injection of various sulphonamides into the udder of cow 1. The two left quarters (A & B) were injected with 10 gm. of sulphone as the acetaldehyde bisulphite derivative on 5.12.44 ; 2 gm. on 8.2.45, and 10 gm. on 15.3.45.
Yield is given in litres (continuous line) ; the dotted line is the *p*H.

but it can be seen from Fig. 3 that, with the exception of the three days during treatment, the milk yield followed the same trend in both groups.

During the work on the behaviour of various sulphonamides and sulphones in the udder their effect on the composition and yield of milk were studied. The results are shown in Fig. 4. Six infusions were made into both right quarters (A and B) and six into both left quarters (C and D) of cow 3 (Herd 6). These injections were made within a period of about six months and on the average they were left in about 18 hours without milking. The results show that they caused some temporary disturbance, but produced no permanent adverse effect on milk yield or *p*H (Fig. 4). Similar results were

obtained in a smaller series of experiments in cow 1 (Fig. 5). In addition, sulphone or one of the sulphonamides was injected into each quarter of cow 4 on five occasions within a period of three months and produced only slight temporary ill effects.

DISCUSSION

In describing events in Herds 1 and 2, the terms " infection " or " recovery " have been used when *Str. agalactiae* was isolated from a quarter from which it had not been recovered at the immediately preceding examination or *vice versa*. It is fully realised that a quarter may be infected when one fails to isolate *Str. agalactiae*. On the other hand, the detection of *Str. agalactiae* in an incubated milk sample may only be due to colonisation of the teat canal ; it would seem important to recognise such teats in work on control of infection and evidence has been presented elsewhere (Francis, 1948b) supporting the value of incubated milk samples. In the present study the isolation of *Str. agalactiae*, or other mastitis streptococci, from the tissue of quarters which had repeatedly failed to reveal these organisms in the milk, provides further evidence that even delicate tests do not always reveal organisms present in the udder. It is rather surprising that five of 25 strains of streptococci isolated from udder tissue were *Str. bovis*, because only seven of 1,778 strains of streptococci, isolated during work on the treatment or control of mastitis, could have been *Str. bovis*. This was not confirmed by testing their power to ferment starch, although the strains isolated from udder tissue were shown to possess this property. During the early stages of work in Herd 1 *Str. bovis* was isolated from ten of 15 vaginal swabs and 19 of 20 faecal samples. It would appear that *Str. bovis*, like *Bact. coli*, must be one of the organisms most commonly contaminating the exterior of the teat, yet both these organisms are very rare in properly taken milk samples.

The recovery of a salicin-fermenting strain of *Str. agalactiae* from three quarters of cow 6, after numerous examinations had indicated that they had recovered from infection, is disturbing. It would appear that cow 1 (Herd 6) also harboured infection for an extended period without *Str. agalactiae* being detected in milk samples. Taken as a whole, the results do not indicate that this type of occurrence is common enough to constitute a serious difficulty in work on the control of *Str. agalactiae* infection, but all statements, based on milk examination, that a quarter has recovered from infection must be treated with some reserve. Even when *Str. agalactiae* was regularly isolated from a quarter there were often no clinical symptoms. In the present study, however, the association of alkalinity, high cell count and clots in the milk with the presence of *Str. agalactiae* confirmed the much more detailed studies of McEwen and Cooper (1947). They showed that infection with *Str. agalactiae* was more likely than any other infection of the udder

to produce a high cell count, and this is probably the most delicate indication of inflammation in the udder (Chu, 1949.)

It has been suggested (Murnane, 1945) that it might be more economical to treat all quarters of all cows in a herd with penicillin instead of carrying out bacteriological examinations. In order to dispense entirely with bacteriological examination, the method of treatment would have to be effective in every instance, making it almost unique. In any event, there would seem to be little justification for treating all quarters in such herds as Numbers 4 or 5, particularly as so much has been written condemning the indiscriminate use of penicillin. The amount of penicillin required would be enormous, and supplies are still inadequate.

Herds 1 and 2 were both heavily infected herds in which " infection " and " recovery " of quarters was frequently occurring, *i.e.*, the position was similar to that in many herds described in Report (1944). Infected cows were milked last and the teat cups dipped in chloros, but no attention was paid to the danger of infection from milkers' hands, dust, or articles in the cowshed, and although this is probably desirable it has been shown that *Str. agalactiae* quickly dies out in infected dust (Spencer, *et al.*, 1946). The results are still on a small scale, but despite the findings of other workers (Report, 1944), it would seem that infection of the teats played no important part in the epizootiology of the infection in these herds, or at least that this infection disappeared when an infection of the udder tissue was eliminated. The successful eradication of infection emphasises the overriding importance of the heavily infected udder as the source of infection, and the value of sulphone, particularly in combination with penicillin, for treating these udders. Before the herds were taken over by us and submitted to bacteriological control, treated quarters must often have become reinfected but when once simple hygienic precautions were taken and the worst quarters were treated, *Str. agalactiae* appeared to spread very slowly.

The findings support the view that mastitis associated with *Str. agalactiae* behaves as an infectious disease, and it has been the purpose of the present investigation to show how the modern therapeutic measures now available may be used in the eradication of the infection. With their aid, and the simple diagnostic methods described elsewhere (Francis, 1948b), it would appear that the accomplishment of this task could be simpler and less costly than the eradication of tuberculosis.

SUMMARY

The eradication of *Str. agalactiae* infection from five herds and observations on the epizootiology of the disease are described and summarised. It was demonstrated that the distribution of infection in the different quarters was not random and that infection with *Str. agalactiae* had an adverse effect on the quality of the milk. The

various therapeutic procedures had only a slight temporary ill effect and their ultimate effect was beneficial.

When first examined the five herds contained a total of 146 cows, 46 of which were infected in a total of 74 quarters. The main facts concerning the eradication of infection are presented.

Satisfactory methods of treatment and control had to be developed whilst working in Herds 1 and 2 and eradication therefore took a considerable time. It was delayed in Herd 3 on account of retention of a cow highly resistant to therapy. In Herds 4 and 5 eradication was rapid.

Eradication depended almost entirely on the use of chemotherapeutic measures ; only very simple hygienic precautions were adopted. In Herds 1 to 5, 40 quarters recovered following injections of 10–20 gm. of 4 : 4′diaminodiphenyl sulphone, 42 following injection with a mixture of 10 gm. of 4 : 4′diaminodiphenyl sulphone plus 50,000 or 100,000 units of pencillin, and six following various experimental therapies. To achieve eradication in each of four herds, one cow with an infected quarter resistant to therapy had to be sold. If the results obtained by Berger and Francis (1949) are included, 50 of 63 (79·4 per cent.) dry quarters and 20 of 33 (61 per cent.) lactating quarters treated with a single dose of sulphone recovered. With a dose of 10 gm. of sulphone, plus 50,000 units of penicillin, 59 of 62 (95 per cent.) of dry quarters and 87 of 103 (84·5 per cent.) lactating quarters recovered. In all 146 of 165 (88·5 per cent.) of quarters treated with a single dose of sulphone plus penicillin recovered, which is a better recovery rate than that reported even with multiple doses of penicillin.

On the basis of this work, it is felt that the eradication of *Str. agalactiae* infection from a herd, area or country may be a simpler and less costly task than the eradication of tuberculosis.

ACKNOWLEDGMENT

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